THE PATHOLOGY OF INTRAPERITONEAL BILE INJECTIONS IN THE RABBIT.*

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Sufficient clinical and experimental evidence has accumulated to demonstrate clearly that bile is a toxic secretion. From a casual review of literature bearing on this toxicity, there would seem to be a tendency on the part of investigators to assume that results obtained with the bile of one species of animal were valid when applied to the bile of all other species. Such an assumption would be justifiable if the sole toxic elements in the bile were the bile salts and the bile pigment, the only variation being the variation in concentration of these, with a further possible variation due to differences that may exist in the toxicity of bilirubin and biliverdin. However, if Meltzer and Salant[1] are correct in their conviction that the effect of the bile is not due to the sum of effects produced by the known components, generalizations in regard to bile would not seem valid, and the toxic effect of the bile of each species would constitute a distinct problem.

Certain experiments carried out in this laboratory would seem to indicate the truth of this latter contention, in demonstrating that rabbit bile is much more toxic to the rabbit than the bile of many other animals is to themselves or to the rabbit, if we may rely on the reports in literature. The experiments were undertaken originally to determine the part played by the various factors in the death of an animal, which, following a general *Bacillus coli* septicemia, had succumbed to a perforating suppurative cholecystitis. The results to be reported concern only the effect of the whole bile from the rabbit's gall bladder, when allowed to escape or when injected into the peritoneal cavity of the rabbit. We have

* Received for publication, July 6, 1911.

[1] *Jour. Exper. Med.*, 1905, vii, 280.

not as yet attempted the separation of the bile into its components, nor have we pushed to completion the toxic effects of the bile when introduced intravenously or subcutaneously.

In the series of cases here reported, the abdominal cavity of the rabbits has, as a rule, been opened high in the mid-line, under strict surgical technique, and with ether anesthesia. The gall bladder has been drawn down, and either it has been opened by cutting off the tip or the contents have been aspirated through a fine needle. The bile thus obtained has been allowed to drip on to the peritoneal surfaces, in some cases in the mid-line, over the anterior surface of the stomach, and in other cases into either the right or the left half of the cavity. The wound has then been closed by separate suturing of the peritoneum and fascia and of the skin. The whole period of anesthesia has varied between twenty and thirty minutes. In the later cases, the aspiration method has been used in an attempt to control the dose of bile. The gall bladder may contain from 0.5 to 2.5 cubic centimeters, and simple incision of the bladder allows no opportunity to estimate the amount which escapes. In some cases, the bile of another rabbit has been injected either through the abdominal wall of the recipient, without anesthesia, or through a minute incision of the parietes with the slightest amount of ether anesthesia.

In considering the results of these experiments, there are two features to be noted: the general toxic action of the bile, and the direct pathological effect. It seems apparent from the series that the bile of all rabbits is not equally toxic. The perfectly clear light green bile has seemed somewhat more toxic than the dark bile with much precipitated pigment. We would not wish to imply, however, that our results necessarily show that the toxicity of the bile is dependent upon the amount of pigment in solution or in combination. While we have not, therefore, determined absolutely the minimal fatal dose of bile when injected into the peritoneal cavity, yet our experiments indicate that it lies between 0.25 and 0.50 of a cubic centimeter per kilogram, the latter dose constantly producing a fatal result in less than twenty-four hours. This seems to be about twice the dose necessary to kill when introduced intravenously. Death is apparently due to direct

action of the bile upon the myocardium. The local pathological effects may be very briefly summarized in the statement that bile apparently produces necrosis of every type of tissue with which it comes in contact. The details of this process will be brought out in a consideration of the individual cases.

It does not seem necessary to give complete protocols of all experimental animals. The delineation of the findings in a typical case, with a brief description of the distinctive features of the other cases, should satisfy scientific requirements. The following case may be taken as a type.

Rabbit F. N. xx.—Male, albino; weight 2,250 grams.

January 6, 1911. 2 P. M. Under ether, the gall bladder was aspirated and 1 c.c. of the clear green bile obtained was allowed to drip over the anterior surface of the stomach and into the left half of the abdominal cavity. The edge of the liver was also touched accidentally. Wound closed. Duration of anesthesia twenty-five minutes.

January 7. Animal died during night. Post mortem examination shows a marked ventral subcutaneous, dark, slightly bile-stained, and hemorrhagic effusion extending from the groins to high up on the thorax. Upon opening the peritoneal cavity, the serous surfaces are found smooth and glistening and free from exudate except for a few small flecks of fibrin near the gall bladder wound. There is a considerable amount of bile-stained serous exudate in the cavity. Both pleural cavities also show a serous exudate, that on the right being bile-stained.

The *heart* is very pale and widely distended, both ventricles being filled with soft, dark clots. The *lungs* are air-containing throughout, but both show congestion and edema of the lower lobes, especially the right lung which is also somewhat bile-stained. (The right half of the diaphragm is bile-stained throughout its thickness.) The *spleen* is noticeably enlarged, is of a dark purplish color, and shows opaque greenish spots on its capsule. The *liver* is large, and pale and has, especially on its under surface, large areas of dead opacity, slightly bile-stained. The cut surface is cloudy, with a subcapsular, narrow, opaque line. On the convex surface of the *stomach* there are opaque greenish spots. The splenic end of the *pancreas* is injected, swollen, and opaque in appearance. In the omental, mesenteric, and especially in the peripancreatic fat, there are small opaque dots of from 1 to 2 mm. in diameter. The *kidneys* are large, dark in color, and somewhat mottled. The cortex is increased in width and has an opaque greyish color with a brownish cast. The vessels are injected. The *thyroid* is pale and greyish. The individual acini are not distinguishable.

Microscopical Examination. Heart.—Throughout the left ventricle wall, there are scattered groups and isolated muscle fibers which are hyaline, often vacuolated, with pyknotic nuclei but with no trace of striation to be made out. There is some fragmentation and segmentation of fibers. The vessels are injected and there is slight edema.

Lungs.—Section through the lower right lobe shows congestion, extensive edema, and necrosis of the pleura with a beginning leukocytic reaction.

Liver.—The liver shows in general marked swelling of the cells with fatty degeneration in the mid and central zones of the lobule. The peritoneal epithelium and the three or four rows of liver cells immediately below the capsule show complete necrosis, with beginning leukocytic invasion.

Spleen.—The spleen shows areas of capsular necrosis covered by flecks of fibrin. The organ itself gives the picture of an acute splenic tumor of the hyperemic type, with pigment present in many pulp cells. There is much fragmentation of nuclei in the splenic cells.

Pancreas.—The pancreas shows interlobular edema, some hemorrhage, and slight necrosis of surface acini, with leukocytic reaction. In the section, there are several areas of typical fat necrosis, the fatty acid crystals appearing as sheaves of fine needles in the necrotic cells. In some areas of fat necrosis, a yellowish pigmentation is apparent.

Kidneys.—The convoluted tubules show necrosis of the epithelium, the nuclei failing entirely to stain. The collecting tubules and glomeruli show nuclear stain. Hyaline casts are present and many tubules show fine yellowish granules which give the Prussian blue reaction for iron. There is edema and congestion of the kidney.

Stomach.—The stomach shows necrosis of the outer layers of muscle.

Diaphragm.—The right side shows complete necrosis of the surface and of the muscle. The muscle fibres are much smaller and hyaline and without nuclear stain.

The muscle of the abdominal wall shows a similar necrosis, and to some extent a disintegration of the fibers. The subcutaneous tissue shows edema, with a very slight leukocytic content.

Thyroid.—The alveoli are small and in both lobes are almost entirely free from colloid. Some of the larger alveoli show, on the contrary, a fibrin network and a finely granular albuminous precipitate.

With the formalin fixation, the blood collections in the section show the brownish granules of hemochromogen and hematin.

The general features of this description will apply to some ten other rabbits of the experimental series, which either died within twenty-four hours after the injection or were killed a short time thereafter. The bile-stained peritoneal effusion and subcutaneous edema have been constant and extensive, especially in the acutely fatal cases. The latter has been due either to bile-staining of the edges of the wound at the time of operation or to leakage of bile along the sutures. Necrosis of the muscle fibres of the abdominal wall has been shown by all. The pale, widely dilated heart with hyaline degeneration and necrosis of muscle fibres in its wall has been constant. The surface necrosis of the liver has occurred whenever bile has touched it and has usually been most marked around the gall bladder leak. Fat necrosis has been a constant

finding, usually in association with hemorrhagic necrosis of the pancreas. The kidneys in all cases have shown signs of toxic action. In them there has been constantly marked congestion, minute hemorrhages, degeneration and necrosis of the tubular epithelium, and casts and hemoglobin within the tubular lumina. From the large amount of pigment precipitated from the blood cells by the formalin fixation, and from the hemoglobin staining of tissues, there has evidently been blood destruction which is further indicated by the acute splenic tumor and the pigment accumulations in the splenic pulp cells.

The special features of the post mortem findings may best be brought out by the condensed protocols. The findings here recorded were confirmed by microscopical examination.

Rabbit F. N. 1.—Female, albino. March 17, 1910. Under ether, the bile duct was tied and the gall bladder opened by a wide incision.

March 18, 1910. Rabbit died during night. Post mortem findings in general as in rabbit xx. Degeneration of heart muscle; necrosis of under surface of liver; edema, necrosis, and slight leukocytic invasion of pancreas; fat necrosis.

Rabbit vi.—Male, Belgian; weight 2,500 grams. March 18. Under ether, 0.65 c.c. of bile was aspirated from the gall bladder and allowed to drip over the anterior surface of the stomach in the mid-line.

March 19. Animal found dead. Dilation and degeneration of heart. Congestion of all viscera. Necrosis of under surface of liver; acute hemorrhagic necrosis of pancreas; peripancreatic fat necrosis; hemorrhagic infarction of a loop of intestine due to thrombosis of mesenteric vessels secondary to bile necrosis of walls of vessels; degeneration of kidney; casts; hemoglobinuria.

Rabbit vii.—Male, Belgian; weight 3,000 grams. March 18. During an attempt to aspirate the gall bladder, it was torn and the bile escaped into the peritoneal cavity in the neighborhood of the gall bladder.

March 19. Animal found dead in morning. Cardiac dilatation. Necrosis of liver. Acute pancreatitis in descending limb; bile-stained fat necrosis; necrosis of wall of mesenteric artery; necrosis of kidney cortex.

Rabbit viii.—Female, Belgian; weight 3,000 grams. (Shown at autopsy to be pregnant.)

March 24. The gall bladder was opened under ether anesthesia and bile was allowed to escape into the peritoneum.

March 25. Animal died during night previous. Most extensive edema and ascites; necrosis of liver and of capsule of spleen. Pronounced degeneration of kidneys; hemoglobinuria.

Rabbit ix.—Female, Belgian; weight 2,500 grams. (Also pregnant.)

March 24. Treated as previous rabbit.

March 25. Animal found dead. Cardiac degeneration and dilatation. Pronounced edema and ascites. Necrosis of liver; acute hemorrhagic pancreatitis; marked degeneration of kidneys; hemoglobinuria; casts.

Rabbit x.—Male, black; weight 2,000 grams.

April 1, 1910. 3 P. M. Ether anesthesia of twenty minutes duration. The gall bladder was aspirated, and 0.5 c.c. of light green watery bile was allowed to flow down over the anterior surface of the stomach.

April 2. Animal found dead in morning. Marked exudation in all serous cavities and in subcutaneous tissue. Dilated heart; necrosis of surface of liver; acute hemorrhagic pancreatic necrosis; fat necrosis; degeneration and necrosis of cells of renal cortex.

Rabbit xi.—Male, black; weight 2,000 grams.

April 1. 3:30 P. M. Operation as in rabbit x, except that 0.5 c.c. of dark green bile was obtained, and was allowed to flow into the right side of the abdominal cavity.

April 2. Animal died between 6 and 8 P. M. Marked exudation; dilated heart; acute hemorrhagic pancreatic necrosis in descending limb of pancreas; fat necrosis; necrosis of liver; necrosis of stomach wall.

Rabbit xxi.—Male, albino; weight 2,250 grams.

January 6, 1911. 3:30 P. M. Under ether, the gall bladder was aspirated and 1 c.c. of clear green bile was allowed to flow into the right side of the abdominal cavity.

April 7. Animal found dead. Lesions almost identical with those in rabbit xx (given in full above), but more extensive. Extensive fat necrosis along descending limb of pancreas and in fat above right kidney. Necrosis of kidney tubules.

Rabbit xxv.—Male, albino; weight 2,700 grams.

January 17. The gall bladder was aspirated and 1.75 c.c. of bile were obtained, of which 0.8 c.c. was allowed to flow into the right side of the abdominal cavity.

January 19. Animal killed. General serous and subcutaneous effusion; dilated heart; congestion and edema of lungs; extensive fat necrosis above right kidney; slight fat necrosis along pancreas; necrosis of surface of liver.

Rabbit xxvi.—Male, albino; weight 2,500 grams.

January 17. Through a small median incision made under ether, 0.75 c.c. of bile obtained from rabbit xxv was allowed to drip into the intestinal surface.

January 19. Animal killed. Beginning fibrino-purulent peritonitis, evidently secondary to the extension from the intestine which showed numerous areas of necrosis extending through the whole thickness of the wall and varying from 3 to 5 mm. in diameter. These lay beneath the point of injection. The pancreas was swollen and injected; surface necrosis of liver.

Rabbit iii.—Male, black and white; weight 1,400 grams.

February 26. 1 c.c. of bile was injected hypodermically through the abdominal wall at about the level of the ensiform. Some escaped into the subcutaneous tissue upon withdrawal of the needle.

February 27. Animal found dead. Subcutaneous effusion; necrosis of muscle of abdominal wall; necrosis of under surface of liver; slight serous effusion; dilated, pale heart.

Rabbit A vi.—Male. April 18. (An accidental experiment.) Under ether, the bile duct was ligated just above the entrance into the duodenum.

April 19. Animal found dead. All the signs of bile intoxication; extensive

effusions; dilated heart; acute pancreatitis; fat necrosis; liver necrosis. Examination showed that the ligature had cut through the duct; bile was escaping at that point.

These cases illustrate well the pathological changes following upon the peritoneal injection of a fatal dose of bile. No type of tissue appears immune to its direct necrotizing effect, and even at a distance, as in the heart, the kidney, and the blood cells, we have evidence that after absorption and dilution by the blood, the toxic action of the bile is still powerful. The death of the animal is apparently due to the effect of the bile upon the heart. This remote effect of the bile is rather surprising, as inspection of the peritoneal cavity has given the impression that the marked pathological effects are more or less sharply localized in the region where the bile was injected or where it acted in concentrated form. The bile appears to be very quickly diluted by the serous exudate to a degree at which its direct toxic action is almost lost. The effect of dilution seemed most marked in animals which received sublethal doses of bile. These animals, killed several days or a week later, often gave little evidence of any injury, unless the bile happened to have been brought into direct contact with a very susceptible organ, such as the pancreas or the liver. In that case, scarring and regenerative phenomena gave evidence of the primary injury.

No organ appears more susceptible to the direct action of the bile than the pancreas, as in practically every case in which the bile has come into direct contact with it, we have found the typical picture of an acute hemorrhagic necrosis of the gland. This is perhaps not surprising in view of the important part played in the causation of acute pancreatitis in man by regurgitation of bile into the pancreatic duct. Bile applied externally to the thin, spread out pancreas of the rabbit is brought into almost immediate relation to all parts of the gland.

The relation of the bile to the fat necroses is not so clear, and possibly these experiments do not prove whether the necroses are due to a combined action of bile and the lipase normally found in the fat cell; or whether it is a combined action of bile and pancreatic lipase, liberated by the injury to the pancreas; or even further, whether the bile is at all responsible except in producing a primary

pancreatic injury. Theoretically there seems to be no reason why the first suggestion might not be true. Bile is toxic to the cell and is further an accelerator of lipase. In the experimental animals, it is true, the fat necrosis was most marked near the pancreas. Yet in several cases it occurred in less than twenty-four hours at points more remote from the pancreas than it is found in rabbits in which the pancreatic duct has been incised and the juice allowed to escape into the peritoneal cavity over a period of several days. In other cases, the necroses were found when the injury to the pancreas, if present, escaped both gross and microscopical examination, although in an organ spread over so great an area, microscopical injuries might escape detection. The question is one which further investigation should clear up.

THE CULTIVATION OF TISSUES IN VITRO AS A METHOD FOR THE STUDY OF CYTOTOXINS.*

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Satisfactory methods for the demonstration of cytolytic or cytotoxic substances of an antibody character, other than hemolysins, have not been perfected, and our knowledge of the mechanism of their action is consequently limited. In fact, the question as to whether we may induce in animals the formation of specific immune bodies for the cells of the various organs of alien species is, in spite of much careful investigation by a number of workers, generally regarded as unsettled. The cause of this uncertainty lies obviously in the lack of a suitable technique for pursuing the problem. The majority of the investigators have based their conclusions on gross and histological studies of the organs of animals previously injected with serum containing the hypothetical specific cytotoxin. Metchnikoff, Landsteiner, Delezenne, Bierry, Pearce, Beebe, and others have published reports of such investigations, dealing particularly with the question of specific cytotoxins for spermatozoa, leucocytes, nervous tissue, and the cells of the kidney, liver, pancreas, and thyroid. There is a striking absence of unanimity in the findings concerning specific antibodies for organ tissues, which is hardly surprising in view of the difficulties encountered in the interpretation of what is often a complex anatomical picture, where the sequence of pathological changes can be stated only as a probability.

In a study of the cytolysins of cobra venom, Flexner and Noguchi[1] were able to demonstrate lytic and agglutinative properties *in vitro* by subjecting emulsions of cells of different organs to

* This investigation was conducted under the George Crocker Special Research Fund. Received for publication, August 4, 1911.

[1] Flexner and Noguchi, *Jour. Path. and Bacteriol.*, 1905, x, 111.

the action of the poison. This technique has been applied by a number of investigators in the study of cytolysins and cell agglutinins in immune sera, but in most instances without conclusive results. Von Dungern,[2] however, demonstrated *in vitro* an epitheliolysin in the serum of guinea pigs treated with injections of ciliated epithelium from the trachea of cattle, and Metchnikoff[3] showed similarly the existence of a spermatoxin in the serum of guinea pigs following injections of rabbit testicle. Agglutinins of questionable specificity for various somatic cells have been frequently observed, and their presence is interpreted by some workers as indicative of a cytotoxic action. On this point, however, Pearce[4] says in a recent paper, "Although such substances [agglutinins and precipitins] may be present, they do not, if we are to follow the rules of bacterial immunization, indicate cytotoxic activity, any more than a fluid agglutinating the typhoid bacillus is to be considered as bactericidal."

For the satisfactory demonstration and study of cytotoxins *in vitro,* then, a procedure is necessary which will admit of the direct observation of cells in an uninjured state for a definite period of time. That is, conditions should be such that the result of an injury to the cells, though insufficient to occasion their rapid death or solution, may be made manifest. Furthermore, it is conceivable that lytic properties might be exhibited against dying or dead cells, such as, for example, is seen in an emulsion of liver tissue, while actively functionating cells would be much more resistant to the same destructive agencies. The technique of Harrison,[5] as modified by Burrows,[6] for the cultivation of tissues outside the body, affording as it does an opportunity for the continuous observation of living cells in a culture medium capable of modification in regard to its antibody content, would seem theoretically to meet all the requirements for an exact method for the study of the action of cytotoxins.

[2] von Dungern, *Münch. med. Wchnschr.,* 1899, xlvi, 1228.

[3] Metchnikoff, *Ann. de l'Inst. Pasteur,* 1900, xiv, 1.

[4] Pearce, Karsner, and Eisenbrey, *Jour. Exper. Med.,* 1911, xiv, 46.

[5] Harrison, *Proc. Soc. Exper. Biol. and Med.,* 1906–7, iv, 140; *Jour. Exper. Zool.,* 1910, ix, 787.

[6] Burrows, *Jour. Am. Med. Assn.,* 1910, lv, 2057.

A wide application of the technique in this direction is, however, obviously dependent on the possibility of cultivating *in vitro* the cells of the different organs of the body. It is true that thus far not all of the tissues of warm-blooded animals have been grown successfully in artificial surroundings. That is, while growths have been obtained from practically every organ, in some cases it was apparent that the specialized cells of the organ were neither surviving nor growing.

Burrows[7] in his studies with chick embryos could easily distinguish growths of skin epithelium from those of mesenchymatous tissues. Moreover, he was able to study the development of nerve fibers just as Harrison had done in frog embryos.

Carrel and Burrows[8] described cultures of spleen, thyroid, kidney, peritoneum, bone, and cartilage, using chiefly the tissues of young dogs and cats. In a majority of the preparations, the cells were apparently of connective tissue origin. In a small percentage of the cultures of thyroid and kidney, however, they observed groups of cells whose arrangement and morphology left little doubt as to their epithelial character. In a previous publication,[9] we called attention to the characteristic alveolar and sheet-like extensions of the cells in cultures of mouse carcinoma, in striking contrast to the independent or string-like radial growth of sarcomatous tissue. The type of growth in carcinoma cultures resembles very closely that described by Harrison for the squamous epithelium of frog embryos, and by Burrows for the skin of chick embryos.

It is seen, then, that from warm-blooded animals, nerve fibers, connective tissue, squamous epithelium, the cells of malignant growths of both types, and to a lesser extent the cells of certain secreting glands (thyroid and kidney) have been cultivated outside the body.[10] With the exception of the studies of Harrison[11] and

[7] Burrows, *Jour. Exper. Zool.*, 1911, x, 63.

[8] Carrel and Burrows, *Jour. Am. Med. Assn.*, 1910, lv, 1379.

[9] Lambert and Hanes, *Jour. Exper. Med.*, 1911, xiii, 495.

[10] Two articles containing further data on this question have just appeared. Fleisher and Loeb (*Proc. Soc. Exper. Biol. and Med.*, 1911, viii, 133) describe cultures of the ovary, testicle, and kidney of guinea pigs and rabbits. In the cultures of ovary, there was a preservation of follicles and slight proliferation

Burrows[12] on the development of nerve fibers in cultures of frog and chick embryos, no great effort has been made to cultivate the higher and actively functionating tissues. A sufficient variety of tissues has been cultivated, however, to show that the field is open for the studies that we have suggested.

The experimental work reported in the present paper is concerned with a study of the suitability of plasma from guinea pigs and rats, immunized against certain mouse and rat tissues, as culture media for rat and mouse sarcomata. These transplantable tumors were selected for cultivation because the uniformly satisfactory growths obtainable rendered comparative studies easy. The choice of species for immunization was determined by the results, recorded elsewhere,[13] in the use of different kinds of alien plasma as culture media for mouse and rat tissues. It was found that, of the alien media employed, plasma from rats and guinea pigs gave the best growths of mouse sarcoma, and, similarly, that the best growths of rat sarcoma were obtained in the plasma of mice and guinea pigs.

The results of the studies with each species used for immunization will be recorded and discussed separately.

CULTIVATION OF MOUSE SARCOMA IN PLASMA FROM IMMUNE RATS.

The rats were immunized by means of injections of a "brei" of mouse sarcoma, made by mincing with sharp scissors the non-necrotic portions of fairly young tumors. Injections were made subcutaneously with a Bashford needle. In the following protocol an experiment is described in some detail.

Experiment RM/1A.—A rat weighing 110 gm. received a subcutaneous injection of 0.2 gm. of mouse sarcoma. The injection was repeated twelve days later.

of the epithelial covering. Testicular epithelium survived but did not proliferate. Kidney tubules showed more or less active regeneration.

Lewis and Lewis (*Anat. Rec.*, 1911, v, 277) describe cultures of various tissues of chick embryos in salt solutions. Nerve fibers and intestinal mesothelium presented characteristic types of growth.

[11] Harrison, *loc. cit.*

[12] Burrows, *loc. cit., Jour. Exper. Zool.*

[13] Lambert and Hanes, *Jour. Exper. Med.*, 1911, xiv, 129.

Thirteen days after the last injection, plasma was obtained by bleeding from the carotid according to technique described elsewhere,[14] and fifteen culture preparations of mouse sarcoma were made. A mouse with tumor was kept under ether during this and the succeeding control experiment, and pieces of tissue were excised as needed. In order to avoid chilling and drying of the cells, not more than four cultures were made from each excised piece of tumor. The incubated cultures were examined daily. On the first and second days, the bits of tissue remained quite inactive with marked disintegration in some of the preparations. After three days there was noted in several a wandering out of a few irregularly shaped cells. No further activity occurred.

Experiment RM/1B.—(Control.) Plasma was obtained from a 90 gm. rat by bleeding from the carotid, and fifteen cultures of mouse sarcoma were prepared, the same tumor being used as in the previous experiment. Examination after twenty-four hours showed an active migration of large spindle and polygonal cells. Ring formations with an extension of cells along the cover glass were noted. There was progressive growth during the three succeeding days. A few of the cultures contained active looking cells on the eighth day of incubation.

This experiment indicates that in rats treated with a suitable quantity of mouse sarcoma, there is induced a reaction such that the blood plasma is no longer a favorable medium for the cultivation of the alien tissue. In fact, a majority of the cells in such cultures appear to survive only a short time. It seems probable that the few cells which were seen wandering out after several days were those which escaped the action of the immune bodies. When it is remembered that a culture is composed of a relatively large mass of tissue (0.5 to 1 millimeter in diameter) in a coagulum only ten to twenty times as great, this is plainly not an unwarranted assumption.

Further studies were made with five immune rats with results that did not differ materially from those just recorded. In each case a normal animal was bled as a control, and at least ten culture preparations were made from each kind of plasma. In two experiments the cultures in immune plasma remained entirely inactive. One of the animals used received a single injection of 0.3 of a gram of mouse sarcoma.

It is interesting to correlate these findings with the facts that have been established in regard to the behavior of grafts of mouse tumors in normal and in immune rats. Mouse sarcoma inoculated into normal rats grows actively for eight to ten days, producing a

[14] Lambert and Hanes, *Jour. Am. Med. Assn.*, 1911, lvi, 33.

nodule which is afterwards rapidly absorbed. In rats previously immunized, no tumor nodules are formed. Careful studies by Russell,[15] with a mouse carcinoma, revealed the fact that in immune rats the cells survive only a short time, disintegration being well marked at the end of the third day. He suggested that the histological picture indicated a cytotoxic action. Bashford and Russell[16] later emphasized the contrast between the fate of grafts of mouse carcinoma in immune rats, to which reference has just been made, and the result of similar inoculations in mice artificially immunized by injections of normal mouse tissues. That is, stated generally, with the same immunizing tissue injections, there is a striking difference between the character of the immunity induced in a foreign though closely related species, and the immune reaction (designated "induced resistance to tumor growth") brought about by homologous tissue injections. In the latter case, the cells in grafts, instead of suffering an early death, survive and multiply slowly for eight or nine days, a fact which points to the absence of any specific cytotoxic action. Our findings[17] in connection with the cultivation of rat sarcoma in plasma from rats exhibiting the different types of immunity to tumor growth are, as we pointed out before, in accord with these facts in that they show that such plasma is in no degree toxic for sarcoma cells.

It is seen, then, that investigations of the two types of immunity by *in vivo* and *in vitro* methods have given results which are mutually corroborative.

CULTIVATION OF RAT SARCOMA IN PLASMA FROM GUINEA PIGS TREATED WITH RAT TISSUES.

Cultures of rat sarcoma in normal guinea pig plasma give uniformly good growths. As a rule, at the end of twenty-four hours there is a diffuse radial extension of cells either of a long spindle type, or irregularly shaped cells presenting several processes. There is a slow progressive growth of from four to six days—occasionally

[15] Russell, *Scientific Reports of the Imperial Cancer Research Fund*, 1908, No. 3, 341.

[16] Bashford and Russell, *Lancet*, 1910, i, 782.

[17] Lambert and Hanes, *Jour. Exper. Med.*, 1911, xiii, 505.

of from ten to twelve days. Since guinea pigs easily withstand bleeding, it is possible to test the suitability of an animal's plasma as a culture medium for rat tissues before immunization. In this way results may be carefully controlled.

Sarcoma "brei" and defibrinated blood were the tissues used for immunization. The brei was introduced subcutaneously with a Bashford needle, and the blood injected intraperitoneally.

The following experiment shows the effect of tumor injections.

Experiment GPR/1A.—A 400 gm. guinea pig received a subcutaneous injection of 0.6 gm. of rat sarcoma brei. The injection was repeated nine days later. On the eighth day following the second injection, plasma was obtained by bleeding from the carotid, and fifteen cultures of sarcoma were made. Microscopic examination of these during five days of incubation showed no activity whatever. There was an early disappearance of the blood corpuscles in the preparations. The edges of the pieces of tissue appeared amorphous and granular.

Experiment GPR/1B.—(Control.) Plasma was obtained from a 450 gm. normal guinea pig for fifteen culture preparations of rat sarcoma, the same tumor being used as in the preceding experiment. Observations during the six days of incubation disclosed the usual progressive radial outgrowth of cells.

These experiments show that the plasma of a guinea pig treated with a sufficient quantity of rat sarcoma is unsuitable to the growth of sarcoma cells. That the reaction, however, does not represent the effect of a cytotoxin specific for sarcoma cells is indicated by the following experiment in which rat blood was the immunizing agent.

Experiment GPR/6A.—A 450 gm. guinea pig received intraperitoneally 5 c.c. of defibrinated rat blood. The injection was repeated eight days later. Eight days after the last injection, plasma was obtained for fifteen cultures of rat sarcoma. After twenty-four hours' incubation, the majority of the pieces of tissue looked quite inert. Four preparations showed a slight outgrowth of spindle and irregularly shaped cells, not comparable, however, to any of the growths in the control series of cultures, prepared simultaneously from the same tumor in plasma from a normal guinea pig. Two of the inactive looking cultures showed on the fourth and fifth days scattered shoots of long clear spindle cells proceeding apparently from the centres of the pieces of tissue, an appearance which suggested that the cells farthest removed from the surrounding medium had survived best. Such an interpretation would add further support to the idea of a well defined antibody reaction. We have, nevertheless, occasionally observed this phenomenon in routine cultures, where drying or some mechanical injury through handling the tissue, was the most plausible explanation. These central outgrowths, however, were more frequently encountered in the use of immune plasma.

The immunized guinea pig used in the experiment set forth above was bled again three days later and a second series of cultures was made, with a control series in plasma from another normal guinea pig. The results obtained were practically identical with those just recorded. These studies were repeated further with two more animals similarly immunized, and the findings were equally conclusive with regard to the inhibition of growth in plasma from animals injected with blood.

That hemolysins are formed following the injection of blood-free tissue of any organ into animals of a foreign species, is well known. That, conversely, cytotoxins for organ cells are formed—at least to some extent—after injections of blood, has also been regarded by many as probable. These experiments in tissue cultivation give support to this view.

The appended table gives a record of tests for hemolysins in the serum of rats and guinea pigs whose plasma was used for tissue cultivation. The rat sera were, of course, tested against mouse corpuscles and the guinea pig sera against rat corpuscles. One cubic centimeter of a 2 per cent. suspension of washed corpuscles was used in each tube, and after the addition of the serum, the total quantity was made up to two cubic centimeters. The tubes were incubated for two hours at 37° C., and the results recorded

TABLE I.

Sera.	Dilution.			
	1:5.	1:20.	1:50.	1:100.
	Hemolysis.	Hemolysis.	Hemolysis.	Hemolysis.
Immune rat	+	+	0	0
Immune rat	+/	+	+	0
Immune rat	+	+	0	0
Normal rat	0	0	0	0
Normal rat	0	0	0	0
Normal rat	0	0	0	0
Guinea pig immunized with blood	+	+	+	0
Guinea pig immunized with blood	+	+	+	+
Guinea pig immunized with sarcoma	+	+	+	+
Guinea pig immunized with sarcoma	+	+	+	0
Normal guinea pig	+	0	0	0
Normal guinea pig	+	0	0	0
Normal guinea pig	+	0	0	0

after twelve hours in the ice box. In all cases the serum was not more than six hours old when tested.

It is noted that hemolysins were demonstrable in the sera of all the treated animals. The hemolytic content of the guinea pig sera was generally the greater, as is shown by the results with the weaker dilutions.

Normal guinea pig serum in dilutions of 1 to 5 hemolyzed rat corpuscles, but not in 1 to 20 and higher dilutions. Hemolysis of mouse corpuscles by normal rat serum was not observed in any dilutions.

SUMMARY.

1. Theoretically, the method of cultivating tissues outside the body offers an ideal technique for the demonstration and study of cytotoxins.

2. Mouse sarcoma, which grows vigorously in the plasma of normal rats, shows little or no activity in the plasma of rats immunized by mouse sarcoma injections.

3. Rat sarcoma, readily cultivated in the plasma of normal guinea pigs, either remains quite inactive, or presents a feeble growth in the plasma of guinea pigs previously treated with rat tissues.

4. The inhibition of growth in cultures of rat and mouse sarcomata in plasma from animals of a foreign species immunized against these tissues, is due, in all probability, to the action of cytotoxins.

A STUDY OF THE ELIMINATION OF PHENOLSULPHONEPHTHALEIN IN VARIOUS EXPERIMENTAL LESIONS OF THE KIDNEY.*

By A. B. EISENBREY, M.D.

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The report of Rowntree and Geraghty[1] on the successful use of phenolsulphonephthalein as a clinical test of the functional activity of the kidneys suggested its use in the study of some of the problems of experimental nephritis. It was therefore with the idea of establishing a basis for such use, as well as for its application to the study of the renal lesions of man, that the present investigation was undertaken. The test has been applied to normal animals and to animals with a variety of renal lesions, including "spontaneous nephritis," several forms of experimental nephritis, and conditions due to mechanical injury of the kidney.

METHODS.

The animals used were female dogs which with few exceptions had been kept for two weeks in the department kennels under close observation. Consequently they were in a good state of health and nutrition, and with the exception of three with "spontaneous nephritis," were free from renal disease as far as could be ascertained by routine examination of the urine for albumin and casts.

The technique of the phenolsulphonephthalein test was in general that recommended for clinical use by Rowntree and Geraghty. The test was usually begun within an hour after the animals had received their morning rations with 200 to 300 cubic centimeters of

* Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication, July 15, 1911.

[1] Rowntree and Geraghty, An Experimental and Clinical Study of the Functional Activity of the Kidneys by Means of Phenolsulphonephthalein, *Jour. Pharmacol. and Exper. Therap.*, 1910, i, 579.

water; if done at any other time, a similar amount of water was administered by stomach tube shortly before the test. The dye, one cubic centimeter of the standardized solution (equal to six milligrams of the dry substance), was injected subcutaneously in the thighs, and in prolonged observations, alternate sides were used on successive days. The bladder was then emptied by catheter and the first catheterization for the detection of the appearance of the dye in the urine was made five minutes after the injection. It was early ascertained that in normal animals it appeared, on the average, after a five minute interval. Occasionally it appeared as early as four minutes, but the small amount of urine usually secreted in shorter periods made the five minute interval a convenient and trustworthy average; fully 50 per cent. of the normal animals showed not more than a distinct trace of the dye at this interval, and very seldom was the appearance of the dye delayed for seven minutes. When in the presence of pathological conditions it did not appear in five minutes, the catheter was either left *in situ* or catheterization was done at frequent intervals. It was found that the latter plan was preferable, as the few drops of urine obtained in the tip of a short glass catheter might contain the dye several minutes before the urine would have collected in sufficient quantity to run from the catheter.

When the time of the first appearance of the dye had been thus fixed, the percentage of elimination in the one and two hour periods was determined. At the end of each of these periods, the bladder was catheterized and washed out with boiled and filtered water, and the percentage of the elimination was estimated by the aid of a Duboscq colorimeter, according to the directions of Rowntree and Geraghty. It was found in conditions of low elimination that more accurate readings could be made by diluting the urine of the second hour period to 500 instead of to 1,000 cubic centimeters, and this procedure was followed whenever an elimination below 15 per cent. for a period of one hour was to be expected. Under some circumstances it was found necessary to limit the dilution to 250 cubic centimeters. Only when hemoglobinuria was present has the color of the urine interfered with the estimations. Although no special aseptic precautions have been employed, no local irritation or other

untoward effect has been noted as the result of repeated injections of the drug.

RESULTS.

Normal Dogs.—Tests have been made seventy-nine times on normal animals; of these, twenty-seven were repetitions which were made in order to ascertain the degree of variation, if any, that might be expected under normal circumstances or as the result of changes in diet and environment. The average time of the appearance of the dye was established at five minutes, varying between limits of four and eight minutes. The elimination for the first hour varied from 40 to 60 per cent. and for the second hour from 15 to 25 per cent. The average for the first hour was 50.98 per cent., and for the second hour 21.35 per cent., a total of 72.33 per cent. The twenty-seven tests made at various periods after the first test gave averages for the first hour of 50.55 per cent., for the second hour of 22.59 per cent., and thus a total of 73.14 per cent. As these later tests cover periods up to one week, it is evident that the change to regular cage life and constant diet tended to increase slightly the elimination, probably as a result of a better condition of the animal.

From the above data it is seen that the total elimination for two hours is 60 to 80 per cent. with an average of about 70 per cent., figures which correspond to those of Rowntree and Geraghty for the normal kidney of man.

Spontaneous Nephritis.—Among fifty-five presumably normal dogs used in this study, three cases of "spontaneous nephritis" were found. The urine of each obtained by catheterization contained albumin, and the functional test gave the following results:

TABLE I.

Spontaneous Nephritis.

Dog.	Time of appearance of dye.	Per cent. elimination.			Albumin.
		First hour.	Second hour.	Total.	
5	6 minutes	36.5	30.4	66.9	Large trace.
8	11 minutes	26.3	14.9	41.2	Heavy trace.
41	6 minutes	44.6	8.1	52.7	Trace.

Later tests were made at various intervals, as seen in table II; the animals were kept in the meantime on a diet of dog biscuit with an abundance of water.

TABLE II.

Improvement in Spontaneous Nephritis.

Dog.	Interval.	Time of appearance of dye.	Per cent. elimination.			Albumin.
			First hour.	Secondhour.	Total.	
5	4 days	6 minutes	58.8	18.1	76.9	None.
8	7 days	9 minutes	36.5	16.6	53.1	Trace.
41	5 days	5 minutes	47.0	18.0	65.0	None.

The improvement in the elimination of the dye corresponding to the decrease in the albumin content of the urine is a very striking illustration of one use to which the phenolsulphonephthalein test may be put. The results are analogous to those secured by Rowntree and Geraghty, who found that if surgical patients were given careful pre-operative treatment, when the elimination test was dangerously low, a marked improvement in elimination occurred.

EXPERIMENTAL ACUTE NEPHRITIS.

Uranium Nephritis.—Nine dogs were studied in this series. These, as was the case with all animals throughout the entire study, underwent two or more preliminary tests before the injection of the drug. The only exception to this rule has been in animals which showed an elimination well up toward the high average limit at the first test.

The usual dose of uranium nitrate was 0.015 of a gram injected subcutaneously. Two types of reaction were noted. One type occurred when the animals were relatively small (six to eight kilograms), and was characterized by a fall in the elimination of the dye occurring within twenty-four hours, which averaged in four dogs 34 per cent.; urinalysis and histological study showed a severe grade of nephritis. In the other type, the animals were either larger or possibly less susceptible to the drug. Although albumin was present in moderate amounts, the elimination at the end ot twenty-four

hours was found to have been increased by from 0.8 per cent. to 5 per cent.; in four dogs the average was 2.52 per cent. Later, on the third or fourth day, these animals showed a progressively diminishing total elimination. In the first type, the dye did not appear for seven to eleven minutes, while in the second type it appeared as normally after five minutes. In none of the animals of the first type, even when the total elimination was as low as 2 per cent. or less at the end of the fourth day, was the appearance of the dye in the urine delayed beyond sixteen minutes. The animals of the second type with one exception showed normal elimination time up to the fourth day period, when a delay averaging ten minutes occurred; in the one exception, the delay appeared on the third day. As a rule, animals were chloroformed at the end of the fourth day period, when marked toxic symptoms occurred. In one instance, however, an animal of the second type was tested on the sixth day, and a total elimination of only 2.2 per cent. was found.

The following protocols illustrate the two types as differentiated by the functional tests.

Potassium Chromate Nephritis.—Four dogs were studied in this series. As the animals were used also in metabolism experiments,[2] it was necessary, in order to avoid vomiting, to use small doses of the chromate. In three experiments, 0.03 of a gram was given sub-

TABLE III.

Acute Insufficiency.

Dog 56, weighing 8,600 gm., with urine free from albumin and casts, received 0.015 gm. uranium nitrate subcutaneously.

Period.	Time of appearance of dye.	First hour.		Second hour.		Total.	
		Amount of urine in cubic centimeters.	Dye per cent.	Amount of urine in cubic centimeters.	Dye per cent.	Amount of urine in cubic centimeters.	Dye per cent.
Control	5 minutes	130	61.7	235	16.1	365	77.8
1st day	11 minutes	20	14.2	20	11.1	40	25.3
2d day	10 minutes	26	2.3	24	1.8	50	4.1
3d day	11 minutes	22	1.56	36	1.95	58	3.5
4th day	10 minutes	20	0.97	18	0.85	38	1.8

[2] Austin and Eisenbrey, Experimental Acute Nephritis: The Elimination of Nitrogen and Chlorides as compared with that of Phenolsulphonephthalein, *Jour. Exper. Med.*, 1911, xiv, 366.

TABLE IV.

Delayed Insufficiency.

Dog 86, weighing 7,450 gm., with urine free from albumin and casts, received 0.0075 gm. uranium nitrate subcutaneously.

Period.	Time of appearance of dye.	First hour.		Second hour.		Total.	
		Amount of urine in cubic centimeters.	Dye per cent.	Amount of urine in cubic centimeters.	Dye per cent.	Amount of urine in cubic centimeters.	Dye per cent.
Control	5 minutes	44	60.97	62	17.0	106	78.0
1st day	5 minutes	32	59.52	32	19.23	64	78.8
2d day	5 minutes	58	46.29	38	22.32	96	68.6
3d day	5 minutes	42	29.94	36	14.45	78	44.4
4th day	10 minutes	30	5.8	46	7.02	76	12.8

cutaneously and the dose repeated at intervals of two to three days; in one, the second injection was given at the end of twenty-four hours. Chromium did not cause the preliminary increased elimination characteristic of uranium in small doses. Moreover, although the abundance of albumin in the urine indicated a severe renal disturbance, comparatively little change was found, after small doses, in the total elimination of the dye. In two experiments there was at the end of twenty-four hours a decrease of 5.8 and 14 per cent. respectively, and in a third an increase of 0.9 per cent. A fourth experiment, in which a very severe nephritis was produced, presented a decrease of 30 per cent. In only one of the experiments did the dye fail to appear in five minutes, although in one dog the total elimination had been reduced by repeated injections from 72.2 to 42.5 per cent. After small doses there is, in spite of the severe albuminuria, a marked tendency to rapid recovery of the power to eliminate the dye.

Cantharidin Nephritis.—Nephritis following the subcutaneous injection of cantharidin was studied in four animals. The functional change caused by a large dose, which produced typical hemorrhagic nephritis ending in anuria and death after thirty-six hours, is illustrated in table VII.

A smaller dose produced a mild nephritis which was not sufficient to cause the death of the animal, but delayed the elimination time at twenty-four hours to eleven minutes and caused a reduction in

TABLE V.

Chromate Nephritis of Moderate Grade.

Dog 59, weighing 6,275 gm., with urine free from albumin and casts, received 0.03 gm. potassium chromate subcutaneously, repeated on the third and fifth days.

Period.	Time of appearance of dye.	First hour.		Second hour.		Total.	
		Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.
Control	5 minutes	20	38.88	20	33.33	40	72.2
1st day	5 minutes	24	42.73	19	15.45	43	58.2
2d day	5 minutes	30	28.09	30	18.24	60	46.3
3d day	5 minutes	22	42.56	23	16.78	45	59.3
4th day	5 minutes	38	33.33	22	17.85	50	51.2
5th day	5 minutes	36	25.25	40	20.32	76	45.6
6th day	5 minutes	36	26.04	36	19.53	72	45.6
7th day	5 minutes	50	25.77	32	16.77	82	42.5

TABLE VI.

Chromate Nephritis of Severe Grade.

Dog 96, weighing 5,500 gm., with urine free from albumin and casts, received 0.03 gm. potassium chromate subcutaneously, repeated in twenty-four hours.

Period.	Time of appearance of dye.	First hour.		Second hour.		Total.	
		Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.
Control	5 minutes	20	46.82	22	24.03	42	70.9
1st day	6 minutes	34	22.74	34	18.11	68	40.9
2d day	9 minutes	First and second hour urine combined.				40	3.54

TABLE VII.

Severe Cantharidin Nephritis.

Dog 61, weighing 5,760 gm., with urine free from albumin and casts, received 0.02 gm. cantharidin in acetic ether, subcutaneously.

Period.	Time of appearance of dye.	First hour.		Second hour.		Total.	
		Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.
Control	5 minutes	74	43.86	111	22.12	185	66.00
22 hours	10 minutes	3	3.22	3	5.92	6	9.14

the total elimination of 16 per cent. A tendency to recovery was evident on the following day (table VIII).

TABLE VIII.

Cantharidin Nephritis of Mild Grade.

Dog 77, weighing 5,970 gm., with urine free from albumin and casts, received 0.013 gm. cantharidin in acetic ether, subcutaneously.

Period.	Time of appearance of dye.	First hour.		Second hour.		Total.	
		Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.
Control	5 minutes	20	54.34	38	24.51	58	78.9
1st day	11 minutes	50	39.37	56	23.58	106	62.9
2d day	6 minutes	20	37.31	18	30.12	38	67.4

In other experiments, very small doses produced a renal disturbance evidenced only by a slight albuminuria. Under such circumstances, an increased elimination of about 5 per cent. was observed during the first twenty-four or forty-eight hours, with a return to normal or slightly below normal on the following day. If at this stage the dose was repeated, the elimination was decreased during the following twenty-four hours, but the tendency to rapid recovery remained even after repeated injections of small doses.

Arsenic Nephritis.—The effect of arsenic was studied on two dogs. One received injections of 0.02 of a gram of arsenious acid each day for three days. Except for a temporary fall of 9.2 per cent. after the first injection, the elimination showed very little change until the fifth day, forty-eight hours after the final dose of arsenic, when the excretion of urine was diminished markedly, as was also the total elimination of the dye. At no time during the experiment did the urine contain more than a trace of albumin.

A second animal was given arsenic over a period of four days, and at the conclusion of the experiment, in spite of the fact that the animal was in a very toxic condition and vomited and refused food, there was no albumin in the urine. In this experiment the excretion of urine showed a progressive decrease, whereas the elimination of the dye was slightly increased (table IX).

Diphtheria Nephritis.—The effect of diphtheria toxin was tested on six animals. The dose required to produce a marked renal lesion, without causing the death of the dog within thirty-six hours, was found to be about 0.005 of a cubic centimeter per kilo of body

TABLE IX.

Arsenic Intoxication.

Dog 104, weight 6,590 gm., with urine free from albumin and casts, received 0.02 gm. arsenious acid subcutaneously on two successive days, and 0.03 gm. on the third and fourth days.

Period.	Time of appearance of dye.	First hour.		Second hour.		Total.	
		Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.
Control	5 minutes	80	56.04	22	23.81	102	79.9
1st day	5 minutes	80	66.66	46	15.18	126	81.8
2d day	5 minutes	34	71.73	14	9.76	48	81.5
3d day	5 minutes	13	63.29	14	14.53	27	77.8
4th day	5 minutes	8	72.46	6	13.88	14	86.3

weight for the toxin[3] used. With such doses, a severe nephritis occurred at the end of twenty-four hours, as was shown by the large amount of albumin and numerous casts in the urine. In four experiments, the time of appearance of the dye was delayed and a marked fall in the total elimination occurred. Only one of these animals lived for forty-eight hours, so that only once could the functional test be repeated.

TABLE X.

Diphtheria Nephritis.

Dog 89, weight 8,140 gm., with urine free from albumin and casts, received 0.005 c.c. per kilo of body weight, subcutaneously.

Period.	Time of appearance of dye.	First hour.		Second hour.		Total.	
		Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.
Control	5 minutes	96	64.1	30	14.71	126	78.8
24 hours	6 minutes	16	53.76	16	21.93	32	75.7
48 hours	20 minutes	First and second hour urine combined.				3	3.49

In three other experiments there was, at the end of twenty-four hours, a more marked fall in total elimination averaging 20 per cent., and a slightly greater delay in the appearance of the dye. The

[3] This was procured from the Research Laboratories of the Board of Health of the City of New York through the courtesy of Dr. W. H. Park. Toxicity for the guinea pig was approximately 1/800.

two animals which received very small doses of the toxin showed a slight initial increase in total elimination at the twenty-four hour period.

A comparison of these five well known forms of experimental nephritis shows that renal irritants may cause a slight initial increased elimination of phenolsulphonephthalein which, however, is, as a rule, followed by a decreased elimination corresponding closely to the dosage of the irritant and the degree of kidney injury. The decrease after the injection of potassium chromate, uranium nitrate, diphtheria toxin, and cantharidin is greater than that after the use of arsenic; the latter indeed may lead to severe intoxication without change in the elimination of the dye.

The decreased elimination may occur abruptly on the first day, as after cantharidin (table VII), or on the second day, as after uranium nitrate (table III), or it may occur more gradually. The point of lowest elimination corresponds to the most severe intoxication, and not infrequently is accompanied by evidence of approaching anuria.

Other renal irritants used were crotalus venom and hemolytic and nephrotoxic immune sera.

Venom Intoxication.—Two dogs were tested after the administration of crotalus venom. Doses sufficiently large to produce the lesions typical of this poison are so rapidly followed by death that the use of the functional tests is impossible. With smaller doses the animals fail to show evidence of serious renal involvement, except by the presence in the urine of slight traces of albumin. Under the latter circumstances, one dog, when tested twenty-four hours after injection, showed no delay in the time of the appearance of the dye, but the total elimination had fallen from 74.2 per cent. to 58.9 per cent. A second dog, receiving repeated minute doses of venom during a period of one week, presented no delay in elimination time and only a slight decrease in total elimination from 78.1 per cent. to 74.9 per cent., with a prompt rise to 83.3 per cent. after the administration of venom was discontinued.

Nephrotoxic Immune Serum.—Three dogs received the serum of rabbits that had been injected repeatedly with the cells of the dog's kidney. This serum was injected intravenously in the proportion

of two cubic centimeters per kilo of body weight. Each of the three animals had a marked albuminuria when the functional test was made and each showed an increase of total elimination varying from 6 to 10 per cent. over that of the control period.

Hemolytic Immune Serum.—In these experiments, the serum of rabbits immunized to dog's blood was injected intraperitoneally in doses of 0.5 to 1 cubic centimeter, according to the weight of the dog. Owing to the occurrence of hemoglobinuria, satisfactory functional tests could be made only after the hemoglobin had largely disappeared, usually on the third or fourth day, at a time, however, when albumin persisted. One animal examined at this time showed a total elimination of 38.4 per cent. as compared with 76.6 for the control period; at the same time the appearance of the dye was delayed for sixteen minutes. In other experiments the change was not so great.

These few experiments indicate that the change in functional activity caused by venom and by hemolytic serum can be readily determined by the phenolsulphonephthalein test, thus adding to the number of lesions in which the test is of value clinically.

The failure to find a diminished elimination after the injection of nephrotoxic immune serum was very disconcerting. The lesion caused by this serum is a very definite degeneration of the tubular epithelium, accompanied by an albuminuria of severe grade and by abundant cast formation,—in short, a very typical experimental nephritis. It stands as the only lesion studied in this series which is not characterized by a decrease in the elimination of the test dye, and for this discrepancy no satisfactory explanation can be offered.

RENAL LESION DUE TO MECHANICAL INJURY.

Effect of Prolonged Experimental Anæmia.—To bring about this condition, both renal arteries in three dogs were completely occluded by rubber-protected screw clamps for periods of fifteen, thirty, and sixty minutes respectively. Functional tests were performed on each of the three days following the operation. After occlusion for fifteen and thirty minutes, slight traces of albumin and a few erythrocytes were found in the urine for forty-eight hours after the operation, and there was no delay in the time of appearance of

the dye. The total elimination was increased slightly, from 76.6 per cent. to 82.6 per cent. on the third day in the fifteen minute animal, and from 65 per cent. to 69.2 per cent. in the thirty minute animal.

The effects of the anemia lasting one hour, however, were marked. Albumin, casts, red blood cells, and renal epithelial cells appeared in the urine on the first day, and the animal presented evidence of intoxication, vomited, and refused food. On the third day, these symptoms were less marked. The functional tests are given in table XI.

TABLE XI.

Effect of Renal Anemia.

Period.	Time of appearance of dye.	First hour.		Second hour.		Total.	
		Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.	Urine in cubic centimeters.	Dye per cent.
Control	5 minutes	70	53.19	70	21.36	140	74.5
1st day	8 minutes	14	10.16	15	9.76	29	19.9
2d day	6 minutes	32	13.44	70	13.44	102	26.9
3d day	6 minutes	22	16.44	38	14.20	60	30.6

Reduction of Kidney Substance.—Unilateral nephrectomy was performed on two animals. After a short period of lowered functional activity, there ensues what may be termed a compensatory hyperactivity which reaches its height in about five days and is followed by a return to a lower level in about fifteen days. The time of the first appearance of the dye, however, is not delayed. The effect of successive operations observed over a long period of time is shown in the following experiment.

Experiment XVIII. Normal Dog.—The elimination before operation was 78 per cent.; on the second day after the removal of one kidney, it was 57.3 per cent.; on the 5th day, 84.7 per cent.; and on the 15th day, 68.1 per cent. At this time the upper pole of the remaining kidney was excised and the total elimination forty-eight hours later was 58.5 per cent., and after six weeks 55.6 per cent.; and although the animal was apparently in good health, it was evident that the functional activity of the kidney was less than during the control period.

Rowntree and Geraghty,[4] in a brief communication before the American Society of Pharmacology and Experimental Therapeutics, state that, "in healthy dogs the removal of one kidney is followed by a normal or only slightly lower function for a period of one half to one hour, but that later the function, as indicated by the test, begins to decrease." How late after operation this decrease was found is not stated, but from our experience it is evident that it remains below normal for at least two weeks. We are inclined to consider the continued subnormal elimination, after reduction of the remaining kidney, to be due to the subacute pyelitis which is so common in the dog after partial excision, and which not infrequently causes chronic changes in the renal cortex. We have found such a condition to be not unusual in our partial reduction experiments, and believe that it, and not the reduction itself, is responsible for the low elimination—55.6 per cent. after six weeks—described in the above experiment.

After nephrectomy in man, however, as has been shown by Rowntree and Geraghty, the functional activity, as indicated by the phenolsulphonephthalein test, rapidly returns to normal.

CONCLUSIONS.

The study of a variety of experimental renal lesions in the dog demonstrates that the phenolsulphonephthalein test of Rowntree and Geraghty is one of the most satisfactory and at the same time most delicate methods of estimating the functional activity of the kidney.

The elimination of the test dye is decreased in so-called spontaneous nephritis and in experimental nephritis due to potassium chromate, uranium nitrate, cantharidin, diphtheria toxin, and arsenic, and in those lesions caused by snake venom, hemolytic serum, prolonged renal anemia, and extensive reduction of the kidney substance. Its elimination is not diminished, but is increased, in the presence of the renal lesion caused by nephrotoxic immune serum, and for this discrepancy no explanation is at hand.

[4] Rowntree and Geraghty, Further Data Relating to the Value of Phenolsulphonephthalein in Estimating the Functional Capacity of the Kidney, abstracted in *Jour. Pharmacol. and Exper. Therap.*, 1911, ii, 393.

The test is a reliable method of demonstrating improvement in the functional activity of the kidney, as is shown in our study of spontaneous nephritis.

The increased elimination of the dye occurring after small doses of various irritants, which is frequently characteristic of the early stages of a severe nephritis, and which is seen also several days after unilateral nephrectomy, would appear to have an important relation to the problem of kidney function; and although probably not of clinical importance, it is worthy of further study as a phase of renal activity.

LETHAL CARDIAC ANAPHYLAXIS IN THE RABBIT.*

FOURTH COMMUNICATION.

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PLATES 44–47.

INTRODUCTION.

The general meaning of the term anaphylaxis is now fairly well understood by everybody and it is probably not necessary to go into many details. The term was coined by Richet in order to show that this symptom complex, or rather symptom complexes, was the opposite of immunity. Thus, for example, a guinea pig tolerates an intraperitoneal or intravenous injection of horse serum as if it were salt solution. If the injection, however, be repeated any time after two or three weeks, the animal responds with powerful toxic symptoms, and with proper dosage it usually succumbs. The first injection instead of causing immunity[1] has produced hypersensitiveness or anaphylaxis.

But this remarkable reaction to a second injection of a soluble proteid is limited not only to the guinea pig. The list of animal species which may be sensitized is steadily growing larger, and without hesitation the opinion may be advanced that anaphylaxis represents a fundamental biological phenomenon.

To the scientific interest of this reaction is added the practical moment that anaphylactic phenomena are by no means uncommon in man. Nor are these manifestations always limited to non-fatal complications as in von Pirquet and Schick's[2] serum disease. Gil-

* A preliminary note was published in the *Zentralbl. f. Physiol.*, 1910–11, xxiv, 957. Received for publication, September 5, 1911.

[1] The word is used in its older meaning of protection.

[2] von Pirquet and Schick, Die Serum Krankheit, Leipzig, 1905.

lette[3] has compiled a series of cases from the literature where the injection of antitoxin caused alarming collapse and even death, and similar isolated case records may be found scattered through the medical journals. It is therefore desirable that the functional disturbances which characterize anaphylaxis be investigated, for it is being recognized that the great step forward in therapeutics represented by the employment of sera is accompanied occasionally by some drawbacks. As matters now stand, the practical physician who injects a therapeutic serum sometimes finds that he has brought not life but death to his patient.

This undesirable adventitious action of sera must therefore be neutralized in some way or another, and this obviously can only be done intelligently when the nature of the vital functional disturbance is known. For the very acute anaphylaxis of guinea pigs, where the second injection of serum causes death within a few minutes, the cause of death has been established[4] and one kind of treatment indicated.[5] It might now be assumed that acute anaphylactic death in other species of animals was caused by the same factor as in guinea pigs. It is interesting and instructive that this is not true. A guinea pig, for example, in acute anaphylaxis, dies of strangulation, due to a stenosis in the finer pulmonary air passages. A rabbit, however, which dies of acute anaphylaxis shows practically no sign of pulmonary stenosis and yet its exitus in typical cases is just as rapid as in the guinea pig. In the following pages, it will be shown that acute anaphylactic death in rabbits is due primarily to a failure of the heart muscle to perform its work.

EARLIER WORK ON ANAPHYLAXIS IN THE RABBIT.

Most of the earlier work on anaphylaxis in rabbits deals only incidentally with the acute form of death, and it may be legitimately neglected in this place. The more recent investigators

[3] Gillette, *Therap. Gaz.*, 1909, xxxiii, 159; *New York State Jour. Med.*, 1909, ix, 373.

[4] Auer and Lewis, *Jour. Am. Med. Assn.*, 1909, liii, 458; *Jour. Exper. Med.*, 1910, xii, 169.

[5] Auer, *Am. Jour. Physiol.*, 1910, xxvi, 439. See also preceding articles.

whose work demands attention are Arthus,[6] Wolff-Eisner,[7] Scott,[8] Cesaris-Demel,[9] and Friedberger associated with Hartoch[10] and Gröber.[11]

The earliest as well as one of the best experimental descriptions of serum anaphylaxis in the rabbit we owe to Arthus. He described objectively the most important symptoms of acute anaphylactic death, as well as the more ordinary type which ends in recovery. Among the symptoms which characterize non-fatal anaphylaxis in the rabbit, he described the respiratory disturbance, general prostration, drop in blood pressure, and increased peristalsis associated usually with the expulsion of feces. In cases of acute anaphylactic death in the rabbit, he noted the main characteristics of the blood pressure curve: rapid fall associated with a marked decrease of the amplitude of the beat and abolition of respiratory waves. The only objection to Arthus' work is that his analysis did not go far enough, in short that there is a lack of interpretation of the physiological disturbances which he so clearly describes.

It may not be out of place to call attention to the fact that Arthus' work in anaphylaxis of the rabbit[12] and the dog[13] has not received the attention which it merits. I regret to state that I was not fully familiar with his work on anaphylaxis in the rabbit when my preliminary note on acute anaphylactic death in the rabbit was published,[14] and thus I failed to mention the earliest accurate work on this subject.

In 1904, Wolff-Eisner[15] described briefly acute death in rabbits after the animals had previously received two to four intravenous injections. After a short period of incubation, the animals show a very marked dyspnea, then tonic and clonic convulsions set in, which are followed by death after a few minutes or after hours. Wolff-Eisner rules out the possibility of air embolism, for he obtained the same symptoms after intraperitoneal injections, but he gives no other explanation.

Scott observes after the intravenous injection of serum in properly sensitized rabbits that a certain percentage of the animals die within five to ten minutes after the injection. The main symptoms which he notes are an initial hyperemia of the ears followed swiftly by a marked anemia. Associated with this anemia is marked panting respiration and a rapid, feeble heart beat. Within two or three minutes, the animal lies with legs extended and its head on the floor;

[6] Arthus, *Compt. rend. Soc. d. biol.*, 1903, lv, 817; also more complete in *Arch. internat. de physiol.*, 1908–09, vii, 472.

[7] Wolff-Eisner, *Centralbl. f. Bakteriol., Orig.*, 1904, xxxvii, 576.

[8] Scott, *Jour. Path. and Bacteriol.*, 1910, xv, 33.

[9] Cesaris-Demel, *Gior. d. r. Accad. di med. di Torino*, 1910, lxxiii, 69.

[10] Friedberger and Hartoch, *Ztschr. f. Immunitätsforsch., Orig.*, 1909, iii, 581.

[11] Friedberger and Gröber, *ibid.*, 1911, ix, 216.

[12] Arthus, *loc. cit.*

[13] Arthus, *Arch. internat. de physiol.*, 1910, ix, 179–203.

[14] Auer, *Zentralbl. f. Physiol., loc. cit.*

[15] Wolff-Eisner, *loc. cit.*

the respiration is labored, and the abdomen large and full. There is discharge of fecal matter. In five to ten minutes, convulsions and rapid death may occur. Respiration ceases first, and the heart usually continues to beat for several minutes, and then slows and stops. When the blood pressure was recorded, Scott noted that a fall to 30 mm. of mercury may occur within three or four minutes in the fatal or severe cases. When the respiration stops, there is a slight rise, and then the blood pressure falls to the base level within three or four minutes.[16]

Scott accounts for the prostration and paresis by the profound drop in blood pressure. The fall in blood pressure itself he thinks is due to a rapid and intense capillary dilatation of the portal area, so that the systemic circulation is rapidly depleted into the portal system of blood-vessels. Scott explains this loss of capillary tonus by assuming a sympathetic nerve action or a direct injury of the endothelial cells.[17]

The heart as a possible factor in the production of death is not mentioned.

After my experimental work was completed, an article by Cesaris-Demel[18] was found, in which this author demonstrated unequivocally that the heart of a sensitized rabbit reacts powerfully in anaphylaxis. Cesaris-Demel perfused the excised hearts of sensitized animals with a Ringer-Locke solution to which he added horse serum in concentrations varying from 1.3 to 5.5 per thousand. The solution exerted a distinctly toxic effect upon the normal heart, but the effect on sensitized hearts was considerably more marked. The sensitized hearts responded by a rapid diminution of the amplitude of the beat and by a strong increase in tone so that practically no beats were visible. On perfusing again with Ringer-Locke's solution, the heart resumed its pulsations. The same reaction could be obtained several times, but finally the organ no longer responded but behaved like a normal heart.[19]

Cesaris-Demel obtained similar reactions when the animals were sensitized with bovine serum, egg albumen, and milk. The corresponding antigen was then added to the perfusion liquid. His experiments were carried out in rabbits and guinea pigs.

According to his original article, it does not seem that Cesaris-Demel attempted to find out whether the heart of the intact animal behaved as did the excised one, nor does he give any closer description of the heart during the temporary anaphylactic stoppage.

The work of Friedberger and his co-laborers, Hartoch and Gröber, does not demand much consideration, as far as their experimental interpretation of the functional disturbances of acute anaphylaxis are concerned. The curves and protocols illustrating Friedberger and Hartoch's article[20] do not deal with the acute fatal type of rabbit anaphylaxis, and therefore need no discussion here. In another article by Friedberger,[21] a tracing of blood pressure and respiration

[16] Scott, *loc. cit.*, pp. 33–34.
[17] Scott, *loc. cit.*, pp. 35–36.
[18] Cesaris-Demel, *Gior. d. r. Accad. di med. di Torino*, 1910, lxxiii, 69–80.
[19] Cesaris-Demel, *ibid.*, p. 76.
[20] Friedberger and Hartoch, *loc. cit.*, pp. 630–632.
[21] Friedberger, *Ztschr. f. Immunitätsforsch.*, 1909, iii, 692.

in a passively anaphylactic rabbit is given where the reinjection led to acute death apparently within a few minutes after the injection. This result, however, is utilized by Friedberger only to show that there is a passive anaphylaxis in rabbits.

The third article by Friedberger and Gröber is illustrated by a number of tracings and protocols which show that sensitized rabbits may die acutely after the toxic injection of goat serum. A study of the protocols gives us no information whatsoever regarding the nature of the vital changes involved, and this opinion is expressed by the authors themselves.[22] In fact, this paper may be considered largely as an amplification of work done by Arthus a number of years ago.[23] It must be added, however, that this work of Arthus has escaped the notice of Friedberger and Gröber.

EXPERIMENTS.

Sensitization.—Young rabbits were used which weighed originally less than one kilo. These were sensitized with normal unheated horse serum in a variety of ways: by repeated subcutaneous, peritoneal, or intravenous injections; usually a combination of these routes of injection was used. The dose injected each time varied between 3 and 5 c.c., and the time interval was from five to six days. The number of injections varied between four and eight.

The animals usually showed a well marked loss of appetite and consequent emaciation during the process of sensitization.

Toxic Dose.—The toxic dose of warm horse serum varied between 5 and 20 c.c., and was injected usually into the jugular vein. The time interval between the last sensitizing dose and the toxic dose varied between four and six weeks, though excellent results were also occasionally obtained after six months.

Certainty of Sensitization.—Rabbits are by no means as easily sensitized to a high degree as guinea pigs. While there is no difficulty in so sensitizing rabbits that the toxic injection causes a drop in blood pressure, increased heart rate, and active cecal peristalsis associated usually with fecal discharges, as Arthus[24] accurately described for the first time, there is considerable uncertainty in obtaining an acute lethal exitus. In the various series of rabbits I used, a certain number merely showed the signs described by Arthus and ultimately recovered, while the others died, either within a few minutes or within some hours. If the animals which did not die acutely received another injection of horse serum, this injection, as a rule, produced no effect on the blood pressure or respiration, and the animals behaved as if salt solution had been infused. This state of anti-anaphylaxis has been thought not to exist in rabbits, but the possibility of its production is undoubted and has been observed before this by Scott,[25] Friedberger and Gröber,[26] and others.

The total number of rabbits used was about thirty-five.

[22] Friedberger and Gröber, *loc. cit.*, p. 237.
[23] Arthus, *Arch. internat. de physiol.*, 1908–1909, vii, 487–489.
[24] Arthus, *loc. cit.*
[25] Scott, *loc. cit.*, p. 32.
[26] Friedberger and Gröber, *loc. cit.*, p. 235.

Operative Technique.—The animals were usually anesthetized by ether; only rarely was morphin employed.

For preliminary tests the toxic dose was always injected into an ear vein; during graphic experiments, through a jugular cannula.

The blood pressure was always recorded from the carotid artery by means of a Hürthle spring or membrane manometer. The connecting tubing was filled with 10 per cent. sodium citrate. The mercury manometer was not employed in order to avoid the entrance of fair quantities of anti-coagulant fluid into the circulation during a drop in blood pressure.

When the vagi were cut, tracheotomy was always performed.

Respiration was recorded by a Meltzer pleural cannula[27] connected with a Marey tambour.

When the heart and lungs were merely inspected, the chest was split transversely and held apart by weights. Artificial respiration was given through a tracheal cannula.

When the central nervous system was to be excluded, the medulla was first thoroughly destroyed; then the cord was pithed, through an opening in the lower lumbar region, by a strong wire which was passed upwards into the cranial cavity and allowed to remain *in situ*. Artificial respiration was started after destruction of the medulla.

When the aorta and vena cava were clamped to exclude the splanchnic circulation, laparotomy was performed and these blood-vessels were clamped just below or just above the diaphragm. When done above, the thorax was entered through the tendinous centre of the diaphragm.

In order to prevent loss of heat during the operative procedures, the animals were stretched out on an electric pad.

Controls.—Numbers of normal rabbits received 5 to 40 c.c. of horse serum intravenously without showing any symptoms even remotely resembling anaphylaxis.

RESULTS.

Before giving the results, it should be stated again that this investigation deals practically only with the acute fatal type of anaphylaxis in the rabbit, a type similar in its rapidity to that which may be observed in the guinea pig when the toxic injection is given intravenously.

Symptoms.—In a highly sensitized rabbit, the symptoms of anaphylaxis are as follows: After such a rabbit receives about five cubic centimeters of normal horse serum through the lateral ear vein, very little is to be noticed except perhaps a transitory quickening of the respiration. The animal squats quietly in its box. Within a few minutes, however, the respiration slows and the animal suddenly falls over on its side with a short clonic convulsion.

[27] Meltzer, *Centralbl. f. Physiol.*, 1896, x, 536.

The head is retracted, the pupils are wide, the iris (in white rabbits), tongue, and gums pale, and in some instances, a few feeble cries are heard. There is no respiration. Palpation of the chest shows the heart beat to be very weak or absent. After a little time, a short group of respirations appear, which swiftly grow weaker and finally disappear. The animal is dead. (See figure 1 for a graphic record.)

Autopsy.—Immediate examination shows the animal to be perfectly limp. The abdominal muscles are pale and relaxed; they contract on stroking the peritoneal surface. The peritoneal cavity contains no excess fluid. The gut is usually pale, though the larger veins are visible. There is slight or no peristalsis. The mesenteric veins are very full and the arteries are seen as thin red threads. No hemorrhages are visible in the gut or stomach. The liver and kidneys are dark and full of blood.

On opening the chest, the lungs collapse well, but not fully. The heart is fairly dilated, especially the right auricle and right ventricle. The auricles usually beat swiftly and regularly, but feebly. The ventricles either do not contract at all or only feebly, and at a much slower rate than the auricles; at times hemorrhages may be seen on the ventricular surface. Stimulation of the heart ventricles by stroking, pricking, or faradic current causes only slight or no contraction. On excising the heart, the blood is fluid, and there are no clots or fibrinous masses in the heart chambers, the pulmonary artery, or the aorta. The endocardial surfaces of the ventricles usually show subserous hemorrhages of variable extent. Stimulation of the cut surfaces of the ventricles is sometimes feebly successful on the left side, but only rarely on the right. The right ventricular muscle shows, as a rule, a peculiar soft, paper-like stiffness as it folds when the fluid blood is removed; its color is gray and the muscles may be almost as resistant to the finger nail as connective tissue. The left ventricle is fairly well contracted; its color is reddish brown and its muscle can easily be scraped by the finger nail.

On excision of the trachea and lungs, the latter look faintly mottled and the surfaces and borders show numerous areas of elevation; closer inspection shows that these hillocks are composed of large distended air sacs easily visible by the unaided eye; they are areas of emphysema. Occasionally slight hemorrhages are seen on the surfaces of the lung. From the cut surface of sections, pressure brings a moderate amount of air and more or less fine foam, as if there were some slight pulmonary edema.[23]

The trachea looks bluish, and on slitting it open the mucosa is always found to be strongly congested; this congestion extends into the pulmonary bronchi.

The blood is usually very dark and its coagulation delayed. The clot which forms after thirty minutes to two hours or longer is never firm.

This autopsy may be considered typical of the usual findings in acute anaphylactic death in rabbits.

[23] Also noted by Scott, *loc. cit.*, p. 40.

Finer Analysis of Symptoms and Signs.—The clinical picture and autopsy findings given above obviously point to two organs whose failure separately or collectively might be the immediate cause of death,—the heart and the lungs. To get further insight, therefore, an animal was prepared so that the heart and lungs could be inspected. This was done by splitting the chest transversely and giving artificial respiration. This experimental arrangement not only permitted observation of the two organs, but also served to differentiate which of them caused death. Shortly after the injection of five cubic centimeters of horse serum into the jugular vein, followed by two cubic centimeters of Ringer's solution to wash clear the cannula, the lungs did not collapse as well during the expiratory phase of artificial respiration. At the same time the heart slowed and the right ventricle appeared fuller and tenser than before. A few minutes later, inspection showed a few small hemorrhages on the right ventricular surface. About thirty minutes after the injection of the horse serum, the heart suddenly slowed considerably, the right auricle apparently did not contract at all and the right ventricle filled up strongly and stopped. During this time, the lungs were expanding and collapsing quite well under artificial respiration. During the cardiac stoppage, the animal made a few convulsive movements and the heart began to beat again, but now the ventricles beat only very weakly in groups of two, the second contraction being stronger than the first. The right auricle now beat four times faster than the ventricle.

At autopsy, the abdominal organs showed the picture described earlier in this paper.

The right ventricle was full and distended with blood; the left ventricle showed a moderate tonus. The ventricles contracted very feebly occasionally; both auricles were beating slightly, but much more rapidly than the ventricles. Unfortunately no notes were made in this experiment regarding the irritability of the heart muscle, except the statement that no contraction occurred on cutting open the heart chambers. The heart contained fluid blood and showed no coagula.

The blood was dark and still fluid ninety minutes after autopsy.

The lungs showed typical areas of emphysema on their surfaces and borders, as was described before.

This experiment pointed unmistakably to the heart as the cause

of death, for the lung functioned sufficiently well to be excluded in this instance as a very important factor in the production of anaphylactic death. (See also figure 3 for a graphic record.)

Peripheral or Central Action of the Horse Serum.—While the evidence so far submitted all indicated that the heart was at fault, the data are not conclusive that this organ is primarily responsible for acute anaphylactic death. It might, for example, be assumed that the stoppage of the heart was due to nervous influences from the medulla through the vagi and accelerator nerves. Again it might be assumed that dilatation of the splanchnic blood-vessels played a vital part, especially as Biedl and Kraus[29] have shown that splanchnic dilatation due to a peripheral vasomotor paralysis was the most important action of anaphylaxis in dogs. To answer these questions, a number of experiments were carried out in rabbits whose basal portions of the brain, medulla, and spinal cord had been destroyed by pithing; in addition, the thoracic aorta and vena cava inferior were clamped in order to exclude the splanchnic circulation. Figure 5 gives a graphic record of such an experiment under artificial respiration. Examination of the blood pressure curve shows that the blood pressure rose shortly after the injection of five cubic centimeters of horse serum into the jugular vein from thirty millimeters to eighty millimeters of mercury, the pulse pressure increasing moderately at the same time; then the blood pressure gradually sank, the pulse pressure decreasing markedly. Four minutes after the injection of the horse serum, the blood pressure record shows no heart beats. This experiment demonstrates that the heart itself is the vital cause of death in acute anaphylaxis in rabbits. On immediate autopsy, the heart was found to be full of fluid blood; there were no beats; a prick with the needle or faradic stimuli caused no ventricular contractions. The lungs showed the areas of emphysema always found; the tracheal mucosa was strongly congested. The blood was dark, and a sample taken above the clamp in the thorax did not coagulate during thirty minutes, while a similar specimen taken from the abdominal vena cava clotted in fifteen minutes.

[29] Biedl and Kraus, *Wien. klin. Wchnschr.*, 1909, xxii, 366.

Action on Cardiac Nerve Endings or Cardiac Muscle.—In the preceding sections, it was demonstrated that the toxic injection of horse serum in rabbits affects the heart so that this organ swiftly fails to perform its work. The question arises now whether this is due to an action on the cardiac nerve endings or upon the heart muscle itself or perhaps upon both structures. The evidence at my disposal does not permit any statement regarding a possible effect on the nerve endings, but there are, on the other hand, quite conclusive data that the heart muscle itself is affected. It has already been stated before that the heart of a rabbit which has died of acute anaphylaxis, responds only feebly or not at all to mechanical or faradic stimulation, the tests being performed immediately or within a few minutes after cessation of respiration. It has also been stated that the right ventricle usually shows a definite change in consistency. These two facts show clearly that the heart muscle itself has undergone a profound functional and anatomical alteration, due to the toxic injection.

Nature of the Change in the Heart Muscle.—What this vital change in the cardiac muscle was, formed an annoying puzzle for some time. The solution was indicated numerous times by the observation that the phrenic nerves lost their irritability very soon after the death of the animal, and that the diaphragm itself swiftly failed to contract to direct faradic stimuli, even with the coil pushed home. In addition, it was noticed that the diaphragm looked crinkled and grayish, and felt stiffer than normally to the touch. The significance of all this was not appreciated until it was noticed that an anaphylactic rabbit, whose death did not occur until about one hour after the injection of horse serum, showed on passive flexion a definite stiffness of the hind legs at the hip and knee. It must be added that this animal was still alive at the time, with a weak heart beat and a blood pressure of about twenty-five millimeters of mercury. Immediate examination of the thigh showed that the white muscles looked grayish and opaque, did not react to direct faradic stimuli (0 coil distance), and that the cut surface of these muscles was acid to litmus paper moistened with a 0.9 per cent. saline. These muscles, therefore, were in typical rigor while the animal was still alive. After the death of the animal (cessation

of respiration), the diaphragm also showed the typical signs of rigor: increased firmness, loss of contractibility, and acid reaction to litmus paper. It now became evident that the changes seen in the heart after acute anaphylactic death were also of a rigor type. These changes are especially well shown, as a rule, in the right ventricle, as mentioned before; the right ventricular wall looks grayish and opaque and falls into peculiarly soft yet stiff parchment paper-like folds when the contained blood is removed; mechanical or electric stimulation yields little or no contraction; on testing its consistency, the right ventricle shows an increased toughness, so that in marked cases the muscle resists the finger nail like connective tissue.

The changes described above in the right ventricle are by no means pronounced in the left. In typical cases, the left ventricle shows a moderate tonus; its muscle looks fairly normal in color; it responds to stimulation usually in the same way as the right ventricle, but it was often noticed that a faint contraction could be obtained from the left ventricular muscle when the right was perfectly unirritable; the left ventricle usually shows but little change in consistency when tested with the finger nail shortly after death. About fifteen minutes after cessation of respiration, the left ventricle was firmly contracted to a little gray teat. It is thus seen that while in typical cases the right ventricle shows practically all the signs of rigor shortly after the cessation of respiration, the left ventricle shows practically only one,—loss of irritability on direct stimulation. This fact that the right ventricle passes into full rigor sooner than the left is interesting because it is a reversal of the usual course in post mortem rigor. Joseph and Meltzer[30] have shown that post mortem rigor sets in later in the right ventricle than in the left, when cats, dogs, and rabbits are bled to death. Their data, unfortunately, are not as complete for rabbits as for dogs and cats, but the facts they furnish make it very improbable that rabbit ventricles do not behave in the same way (see especially their tables on pages 20, 24, 28, and 29). This is, therefore, additional proof that cardiac anaphylactic rigor in the acute fatal cases is probably not a *post mortem* but an *intra-vitam* phenomenon, and

[30] Joseph and Meltzer, *Jour. Exper. Med.*, 1909, xi, 32.

further evidence is given by those cases where the heart stops suddenly (figure 3).

In summing up the last section, it may be said that the change in the cardiac muscle which causes death in acute anaphylaxis is probably a rigor. This rigor may be classed as a chemical rigor.[31]

Blood Pressure.—With the animal prepared as described on a preceding page, the blood pressure and respiration were recorded in numerous experiments. The blood pressure curve is fairly characteristic and is as follows: After or even before the toxic dose of warm horse serum has been completely injected into the external jugular vein, the pressure begins to rise, the respiratory oscillations disappear, the pulse pressure increases, and the heart slows moderately. The rise usually does not exceed twenty millimeters of mercury, but it may at times exceed this considerably; it usually does not last longer than one minute, and then the blood pressure falls gradually, the pulse pressure usually decreasing swiftly while the heart rate increases. From now on the pressure curve takes one of two courses: (a) The pressure continues to sink. Within another minute, the twenty millimeter level is reached, but at the same time the pulse pressure again increases; usually the terminal group of respiration occurs during this stage. The increase in pulse pressure is accomplished by another marked temporary slowing of the heart rate. From now on the pressure keeps on gradually sinking while the heart beats become smaller and still more rapid. Three minutes after the injection, the curve shows a pressure of about ten millimeters, and the pressure waves are hardly discernible. Six minutes after injection, the curve is a straight line. Figure 1 gives a graphic record of this type of blood pressure curve. (b) The second type is illustrated by figures 2 and 4. The fall of blood pressure suddenly slows, and at the same time there is a marked increase in the rate and a striking diminution in the size of the pulse pressure. The pressure may remain stationary for a short period, but then slowly drops so that five to ten minutes later the pressure curve has reached about the ten millimeter level. Dur-

[31] See Meltzer and Auer, *Jour. Exper. Med.*, 1908, x, 48–49, for a description of the various kinds of rigor.

ing the final drop the pressure waves become still smaller and are often irregular in size and rhythm (figures 2 and 4).

At times the heart may stop abruptly after the toxic injection; figure 3 gives an example of this. This animal had received three milligrams of curarin and the vagi were intact. With an average blood pressure of about seventy millimeters, there were a few dropped beats and then the heart stopped suddenly and the blood pressure sank to the twenty millimeter level within a few seconds. The curve now shows only a few irregular spasmodic waves, and within two minutes after the drop, only the oscillations due to the artificial respiration are recorded.

This sudden cardiac failure was observed a number of times, and always in rabbits with intact vagi. It might, therefore, be assumed that the stoppage was due to a stimulation of the cardiac vagus endings. This explanation alone, however, is not sufficient, for a permanent cardiac stoppage by vagus stimulation cannot be obtained in mammals, at least by artificial means. Another factor probably enters into consideration, and that is a sudden complete functional failure of the heart muscle. Additional evidence for this is furnished by the behavior of the ventricles of these animals to direct stimulation, which has already been described.

When the influence of the central nervous system, as well as the splanchnic circulation is excluded, as described previously, the blood pressure exhibits greater regularity. Figure 5 gives an example of this. In this animal the blood pressure naturally was low, about thirty millimeters of mercury; but the heart beats were regular and the respiratory oscillations are well shown. After the injection of the horse serum, the pressure rose in about one minute to eighty millimeters, the pulse pressure increasing, and the heart beating more rapidly (from 220 to 310 per minute). The respiratory oscillations were abolished during this rise. After another minute, the pressure began to sink slowly and, accompanying this drop, the pulse pressure rapidly decreased, until four minutes after injection the pressure is about twenty millimeters, and the record shows only a few irregular heart contractions. Five minutes after injection, the pressure curve shows only the oscillations which are produced mechanically by artificial respiration. This curve shows

well the main affects of lethal cardiac anaphylaxis upon the functional power of the heart.

Respiration.—The respiration was recorded in one way only, by means of an intrapleural cannula. The respiratory record in the tracings therefore represents only the intrapleural pressure variations during respiration, but in the present investigations these pressure variations fairly accurately represent the volume variations of the lung. That this is true has been shown in a previous section where the lungs were inspected during acute anaphylaxis and where it was shown that the toxic injection of horse serum exerts only a moderate influence upon the collapse and expansion of the lung under artificial respiration (see figure 3 for a graphic record).

In a typical case of acute lethal anaphylaxis, the respiration behaves as follows: Shortly after the horse serum has been injected and during the initial rise of blood pressure, the respirations decrease in amplitude and either quicken or slow slightly, depending apparently upon the respiratory rate preceding the injection (see figures 1 and 2). There usually are a few short, sharp struggles. Now the respirations begin to slow and the amplitude increases considerably; during this stage, which lasts a number of minutes, the blood pressure falls to a low level. This stage of slow, deep, respirations may be terminated by tonic and clonic convulsions followed by a respiratory stoppage (figure 1), or the stage of slow respirations is broken by a number of short sharp convulsive struggles, after one of which the respiratory pause appears (figure 2). When the respiratory stoppage occurs which takes place two to five minutes after the serum injection in highly sensitive animals, the blood pressure is still falling gradually and shows abrupt changes in the rate of beat. These changes in the rate of beat show the establishment of a block, for the respiratory tracing records three or four auricular contractions before the ventricles contract (see figure 1). The respiratory stoppage lasts about one half minute and then the terminal group of swiftly decreasing respirations appears (figures 1 and 2). Respiration now ceases permanently, but the heart is still beating very feebly and the blood pressure has practically reached its lowest level. There is no recovery from this stage.

When the volume changes of the lung during artificial respiration are recorded indirectly by means of a pleural cannula, the animal being immobilized by curarin, no changes are noticed in the record during acute anaphylactic death (see figure 3). This indicates that no very marked stenosis of the pulmonary air passages occurs in rabbits during acute anaphylaxis; surely none which could in itself cause death.

The respiratory changes are due probably to influences upon the respiratory centers. Whether this is merely the effect of a failing blood supply, for the alteration in respiration sets in about the same time that the heart begins to slow (figure 2), or whether it is due to a hypersensitiveness of the respiratory nerve centers cannot be determined from my experiments.

Effect of Some Drugs.—I have only a few experiments regarding the effect of drugs upon the development of acute lethal cardiac anaphylaxis, This section of the work was hampered by the fact that I never could feel certain that the animal would succumb acutely. This necessarily excluded the use of prophylactic injections, for if the animal survived there was no guarantee that recovery was not due rather to a relatively low sensitiveness than to the protection of the drug. I was therefore compelled to inject the drugs during the terminal stages when the heart was already surely damaged.

The drugs used were digalen and strophanthin. Both were injected intravenously during the terminal stages usually, and both exerted apparently a distinctly harmful effect. This was especially noticeable when, for example, strophanthin (Thoms) was injected during the time when the blood pressure was still good but when the heart beat was very rapid and the pressure oscillations small. Under these circumstances, the pressure rose moderately and the heart increased still more in rate for a short period of time; then the pressure sank rapidly and very soon no beats were recorded. I received the impression that this injection directly caused death. While these few experiments are insufficient to permit definite statements, there is enough evidence to urge great caution in the employment of cardiac stimulants (digitalis group) in cases of cardiac anaphylaxis.

DISCUSSION.

In the preceding sections, experimental facts have been described which show that in acute lethal anaphylaxis of rabbits the fatal outcome is primarily due to a failure of the heart to perform its work. It was also shown, by destroying the central nervous system and excluding the splanchnic circulation, that the cause for this failure resided in the heart itself. This was still further strengthened by demonstrating that the heart itself immediately after cessation of respiration, responded only feebly or not at all to mechanical and electrical stimuli, and that there was also as basis for this functional change, an alteration of the anatomical properties of the heart muscle which was especially marked in the wall of the right ventricle. This anatomical change was interpreted as a rigor of the heart muscle, because this muscle showed a marked change in color, translucency, consistency, and irritability.

This work, therefore, agrees with the interesting research of Cesaris-Demel which has been described previously. I may be permitted, however, to point out that Cesaris-Demel limited himself to the demonstration that the isolated heart was affected in anaphylaxis; he did not show that this also occurs in the intact animal, nor did he study the nature of the cardiac change produced in anaphylaxis.

The question might now be asked, how it was known that these animals were really anaphylactic. This is easily answered. In the first place, the largest dose of horse serum injected was about twenty cubic centimeters, and such a dose is tolerated by normal animals without any obvious respiratory or circulatory effect; any disturbance which arises in a sensitized animal after the injection of the antigen may therefore legitimately be ascribed to a state of hypersensitiveness or anaphylaxis.

Diagnostic Character of the Blood Pressure and Respiration.—Perhaps the most striking characteristic to be noted in the blood pressure curve, when recorded adequately, is the initial increase and then the rapid progressive diminution of the pulse pressure associated with an increasing fall of blood pressure (figures 1, 2, 4, and 5). This characteristic cannot, however, be considered diagnostic of anaphylaxis *only*, for other substances which produce a similar

change in the heart muscle will surely cause a similar blood pressure picture. The blood pressure curve can only be said to be characteristic of a certain cardiac functional alteration. This viewpoint will be considered more in detail later.

The respiratory picture may be regarded in the same fashion (figures 1 and 2); it shows nothing which can be considered characteristic *only* of acute lethal anaphylaxis in the rabbit.

Both blood pressure and respiration may, however, be considered perfectly characteristic of anaphylaxis if the conditions under which they are produced are clearly kept in mind. This point will be discussed more fully in the following section, but before this can be done it is necessary to have a clear conception of what should be understood when the word anaphylaxis is used.

Basic Conditions for the Diagnosis of Anaphylaxis.—The diagnosis of anaphylaxis is at present only justified when the injection of the substance used for sensitization causes symptoms and signs which are not obtained, or at least in a less degree, when the same substance is injected into a normal animal. There is nothing new in this statement, for it is simply a definition of what the word anaphylaxis means. The two fundamental conditions, therefore, are that on first injection a substance may be harmless or only slightly harmful, but that a second injection, after a proper interval, should exert a distinctly harmful effect.[32] These basic conditions for a legitimate employment of the term anaphylaxis have been ignored in much of the recent work. Thus Friedberger's "anaphylatoxin" is misnamed, for this substance, produced by various biological manipulations from normal sera, causes death in Friedberger's hands when injected into *normal* guinea pigs. The mere fact that Friedberger claimed that he obtained the lungs characteristic of acute anaphylactic death in guinea pigs with anaphylatoxin[33] cannot be considered legitimate proof that his "anaphylatoxin" causes true anaphylaxis, any more than it can be claimed that barium chlorid and eserin cause anaphylaxis because these substances pro-

[32] It is obviously possible that anaphylaxis might also be beneficial in regard to some functions as yet unknown.

[33] This is vigorously denied by Biedl and Kraus, *Ztschr. f. Immunitätsforsch.*, 1910, vii, 220–221, 408–413.

duce a greater or less approximation to the lung picture found in acute anaphylaxis of guinea pigs. *It must be remembered that the lung condition which Lewis and I described in guinea pigs that die acutely of anaphylaxis is characteristic of this condition only when a sensitized guinea pig is injected intravenously with its antigen.* In other words, the inspiratory immobilization of guinea pig lungs is characteristic of anaphylaxis only when the conditions for its production are fulfilled: intravenous injection of the substance used to produce sensitization in guinea pigs.

These considerations hold good at present for every sign and symptom which have been described in anaphylaxis in any species of animal; in order to be characteristic, they must be obtained in the only manner legal for anaphylaxis: on second injection of the substance used to sensitize. It should be noted that I stated that these considerations hold good "at present." They will not be valid when the real anaphylatoxin[34] has been isolated. But this anaphylatoxin must, in my opinion, fulfill certain conditions: it must be biologically isolated from the blood or tissues of an animal which had a true attack of anaphylaxis, and this substance must not be found in normal animals, but it must produce the typical symptoms when injected into them.

Attention may be called in this connection to another error. It is perhaps not generally appreciated that the symptoms and signs of anaphylaxis differ considerably in the three species of animals which have so far been carefully studied. Thus, for example, the characteristic drop of blood pressure in the dog is not found in the acute cases of anaphylaxis in the rabbit and guinea pig; the large, pale, fully inflated lungs of the guinea pig are not found in the dog or the rabbit; and again the intravital rigor of the heart muscle in the rabbit is not seen in the dog and the guinea pig. There are numerous other differences, but those cited are sufficient to indicate clearly that each species of animal must be measured with its own yardstick when examined for anaphylaxis. It is, therefore, not permissible to apply indiscriminately a characteristic sign of one species to another species, as Friedberger and Gröber do when they

[34] Whether the assumption of an "anaphylatoxin" is necessary or not, I do not care to discuss.

state that the drop of blood pressure seen in acute lethal anaphylaxis in the rabbit cannot be referred to a pulmonary inspiratory rigidity, and that this pulmonary rigidity is not characteristic of anaphylaxis anyway, because all sorts of poisons cause this sign in rabbits and guinea pigs.[35]

The considerations given above clearly show, it seems to me, the necessity of limiting at present the scope of the word anaphylaxis to its original restricted meaning which demanded two injections of the same substance, the first one producing only sensitization, while after an appropriate interval the second injection caused powerful toxic symptoms. Only under these conditions will confusion be avoided, and the diagnostic value of the various signs and symptoms established for anaphylaxis be preserved.

SUMMARY.

Acute lethal anaphylaxis in the intact rabbit is caused by a failure of the heart.

This failure of the heart is due to a change in the heart itself; it is peripheral and independent of the central nervous system for its production.

This change in the heart is shown anatomically and functionally by decreased translucency, change in consistency, and by failure to respond to stimuli, and is probably to be classed as a chemical rigor.

The rigor of the heart is most pronounced in the right ventricle, the wall of which may be gray, stiff, very tough to the finger nail, and non-irritable.

Cardiac stimulants of the digitalis group seem to exert a harmful effect when injected in acute anaphylaxis.

Blood coagulation is delayed; a loose clot forms after one half to two hours.

Anti-anaphylaxis is produced when the animal does not succumb to the injection.

When anaphylactic death is delayed for about one hour, a well developed rigor of the white muscles of the thigh, and of the diaphragm may occur while the animal is still alive.

Reasons are brought forward to show the necessity of more caution in employing the word anaphylaxis.

[35] Friedberger and Gröber, *loc. cit.*, 236.

Friedberger's statement, that the lungs of guinea pigs dead from acute anaphylaxis are not characteristic of anaphylaxis for this animal, is shown to be baseless.

EXPLANATION OF PLATES.

In the tracings which show two curves (figures 1, 2, and 3) the upper curve represents respiration, registered by means of a pleural cannula; the straight line transecting this is the atmospheric pressure line: above the line is positive, below is negative pressure.

The curve with smaller oscillations is the blood pressure curve, written always by a Hürthle spring or a membrane manometer. The straight line below it is zero pressure. The calibration scale at the end of the curve gives the value of the different levels in millimeters of mercury.

Time is marked invariably in four second intervals.

The broad white lines below the time line show the duration of injections, stimuli, etc.

PLATE 44.

FIG. 1. Experiment of December 22, 1910. White rabbit, male, weight 1,200 grams. Sensitized by peritoneal injections of 3 c.c. of horse serum on September 29, October 7 (subcutaneous), 13, and 20. Cannulae in external jugular vein, trachea, left carotid artery, and left pleura.

Downstroke of respiration curve = inspiration. Vagi intact. Toxic dose of horse serum: 5 c.c. into the jugular vein. Further details are given at the head of this section. This tracing gives a good general picture of acute lethal anaphylaxis in the rabbit.

PLATE 45.

FIG. 2. Experiment of May 9, 1911. White rabbit, male, weight 1,660 grams. Sensitized by the intraperitoneal injection of 3 c.c. of horse serum on November 25, 1910, November 30, December 5, and December 10. The operative procedure was exactly the same as in the preceding animal, except that blood pressure was recorded from the right carotid artery. Vagi intact. Toxic dose of horse serum: 10 c.c. into the jugular vein, repeated once. Further details are given at the head of this section. This plate gives a modification of the type shown by figure 1.

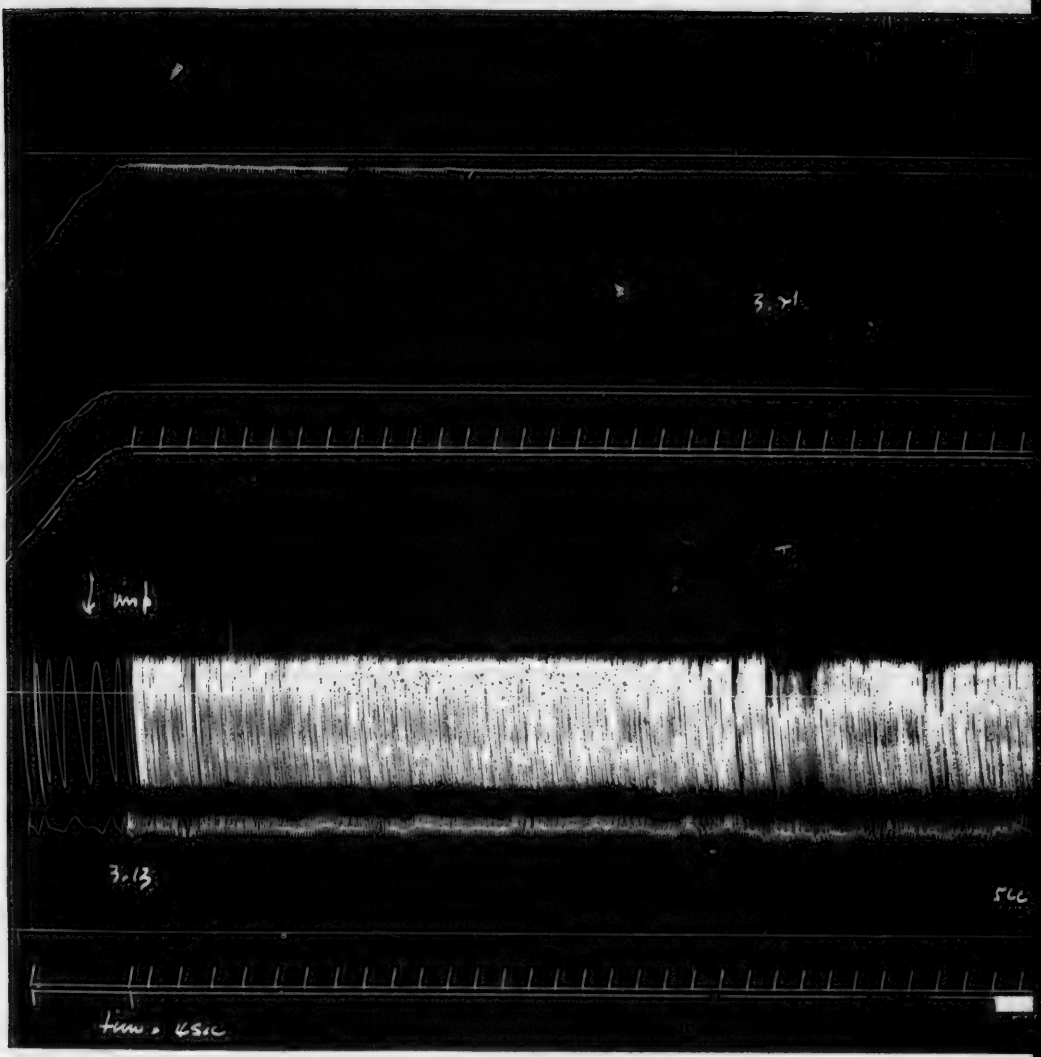
PLATE 46.

FIG. 3. Experiment of March 22, 1911. White rabbit, male, weight 1,760 grams. Sensitized by the intraperitoneal injection of 3 c.c. of horse serum on November 25, 1910, November 30, December 5, December 10, and on February 24, 1911, 2 c.c. injected into ear vein. Cannulae were inserted into the left external jugular vein, trachea, right carotid artery, and right pleural cavity. Three milligrams of curarin given intravenously. Vagi intact. Artificial respiration recorded: upstroke represents inflation of lung. Toxic dose of horse serum: 9.5 c.c. into the jugular vein; after a few minutes, 5 c.c. Note the sudden stoppage of heart beats and the unvarying respiratory amplitudes. This plate shows well how swiftly the left heart may fail.

PLATE 47.

FIG. 4. Experiment of May 10, 1911. Gray and white rabbit, male, weight 2,170 grams. Sensitized exactly as preceding animal. Cannulae were inserted in the external jugular vein, trachea, and right carotid artery. Both vagi cut. Three milligrams of curarin were injected into the jugular vein; artificial respiration. Toxic dose: 10 c.c. of horse serum into jugular vein, repeated once. Note the change in character of the blood pressure curve after the injection.

FIG. 5. Experiment of March 17, 1911. White rabbit, female, weight 2,190 grams. Sensitized by the peritoneal injection of 3 c.c. of horse serum on November 25, 1910, November 30, December 5, December 10, and on January 9, 1911, 2 c.c. into ear vein. Tracheotomy; vagi cut; cord, medulla, and basal portions of brain destroyed by pithing. Artificial respiration. Cannulae into left external jugular vein and carotid artery. Chest opened through diaphragm and vena cava and thoracic aorta clamped. Toxic dose of horse serum: 5 c.c. into jugular vein. Note the rise in blood pressure and the abolition of respiratory oscillations, then the rapid decrease of the pulse pressure during the drop in pressure, and the development of irregularities and cessation of the heart beat.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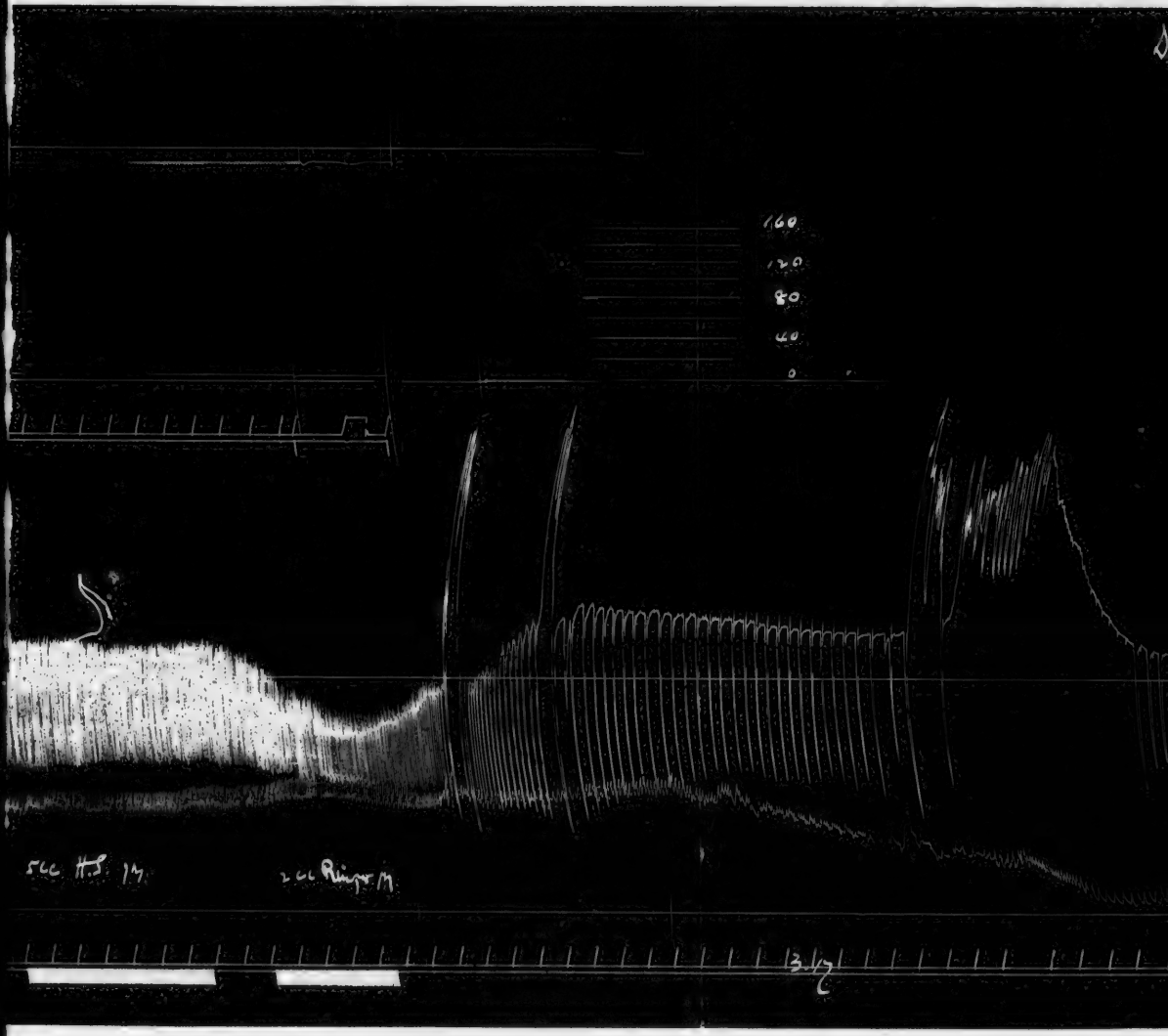

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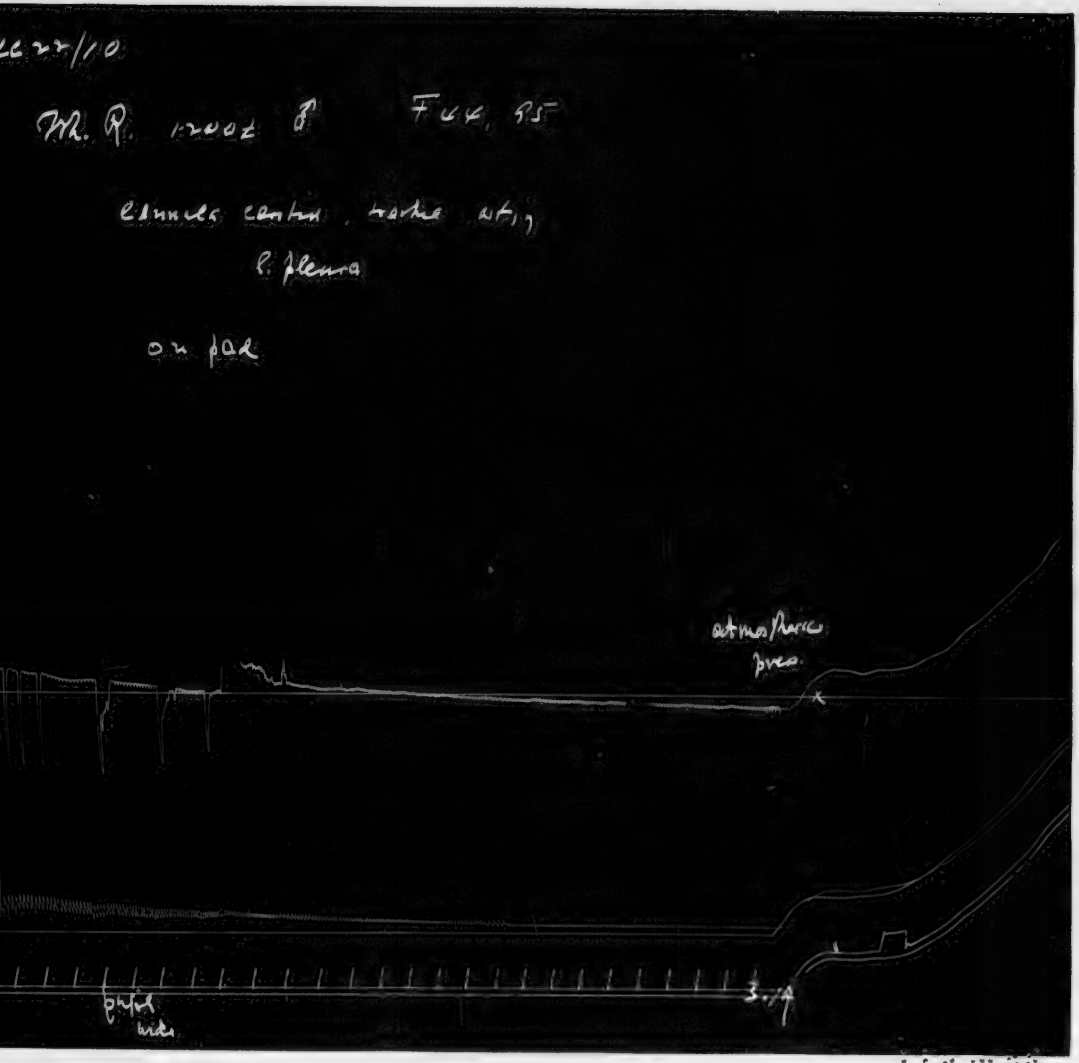
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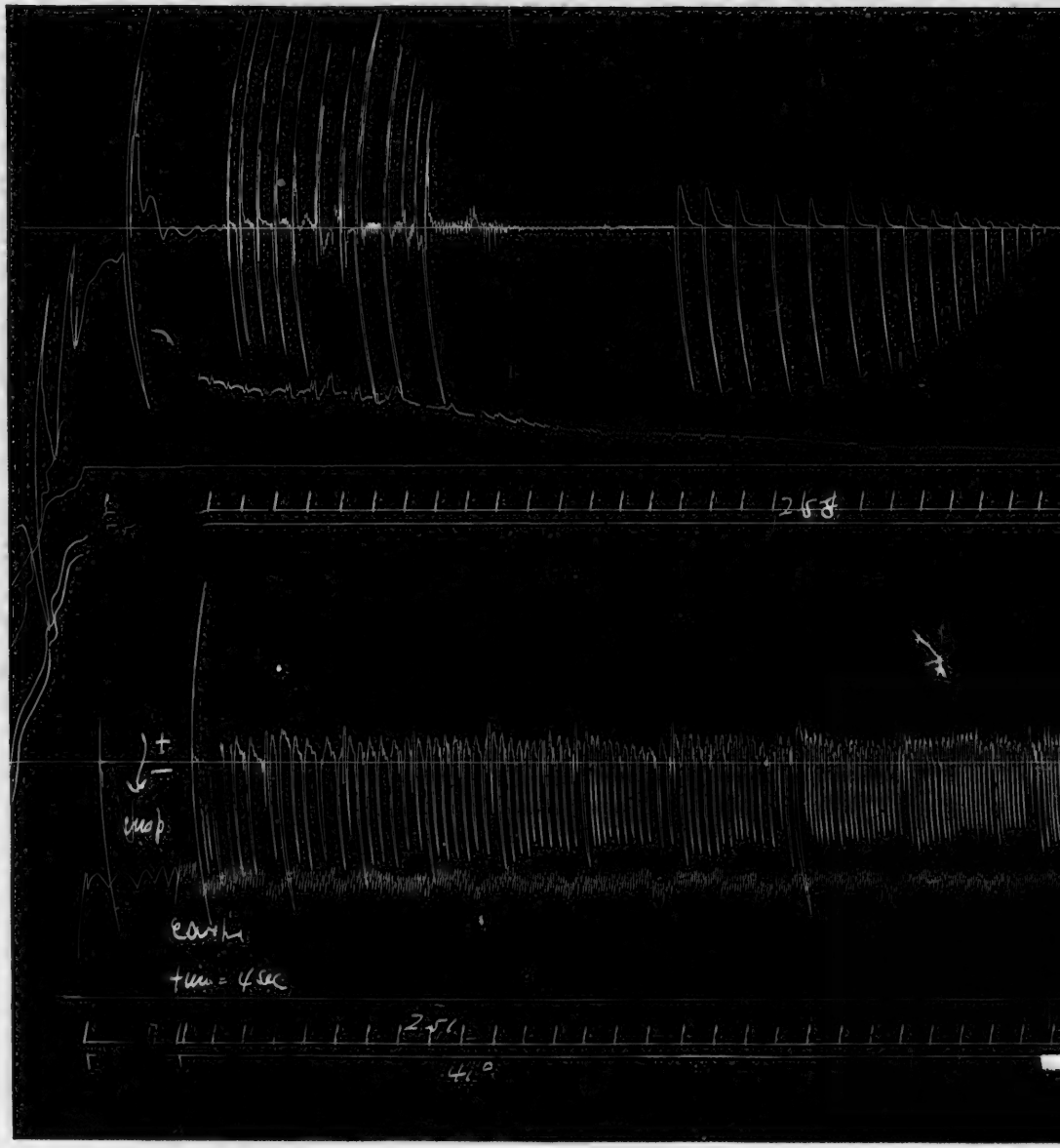
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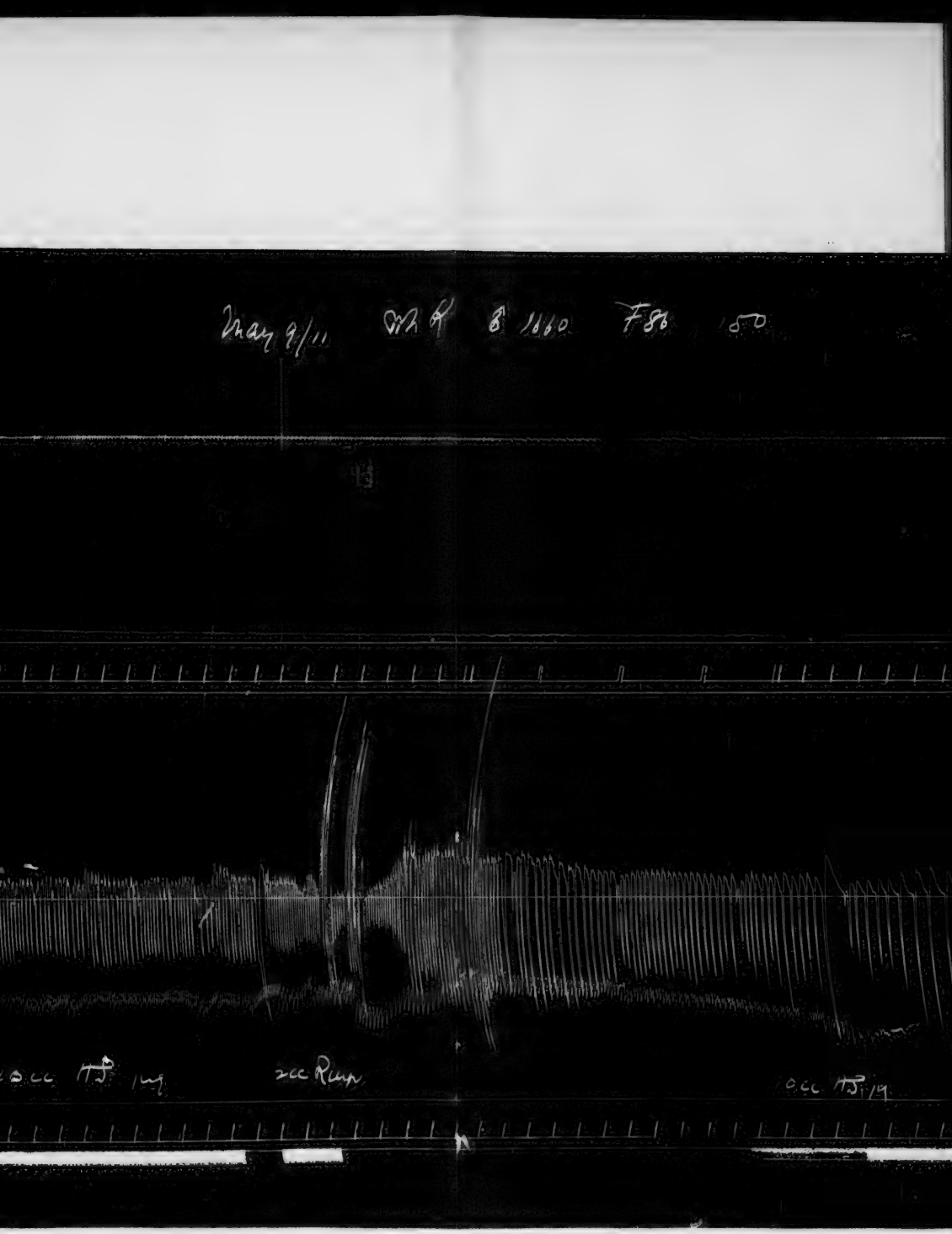

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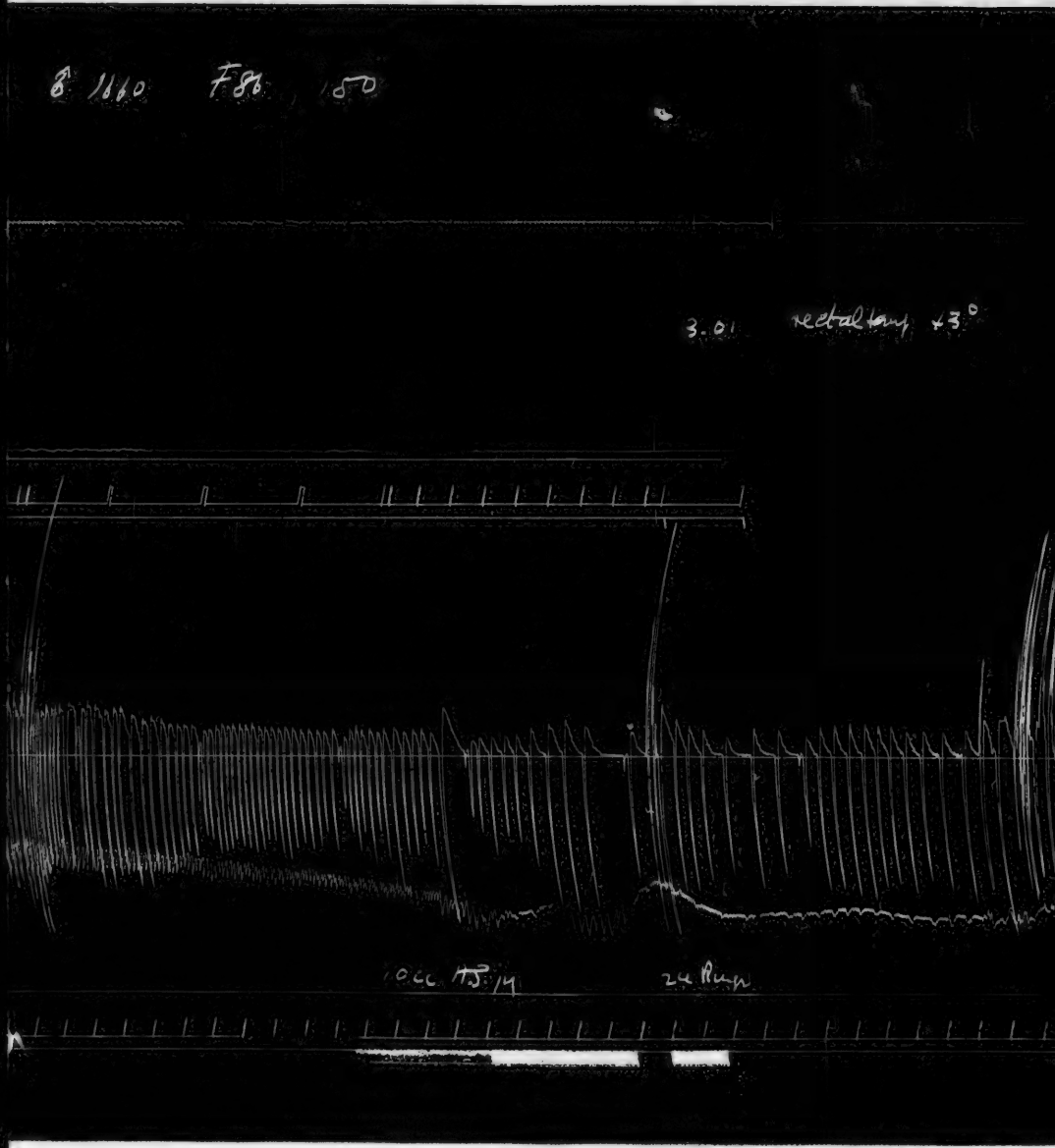
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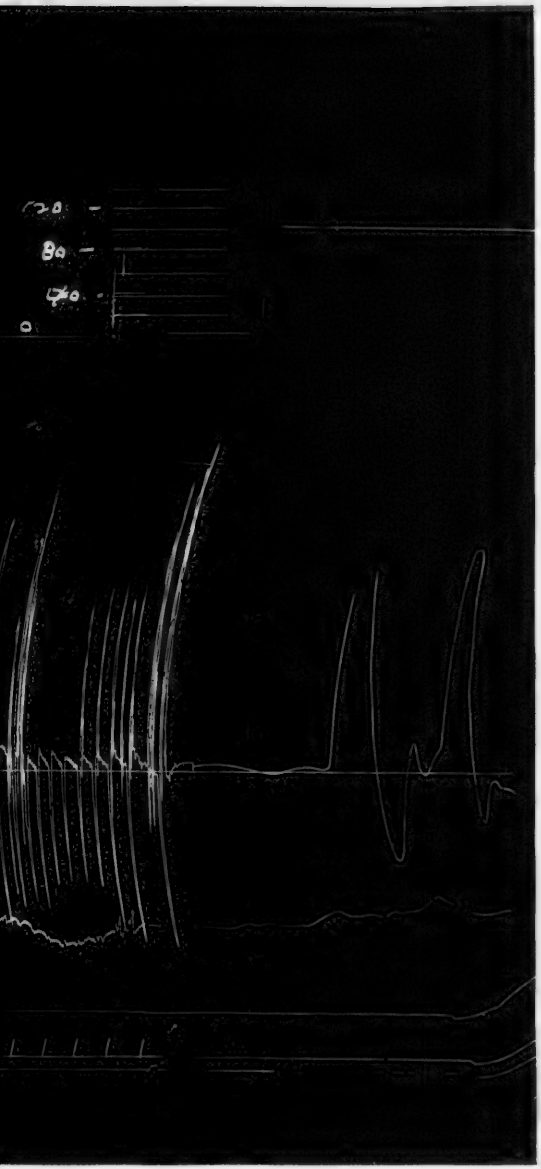
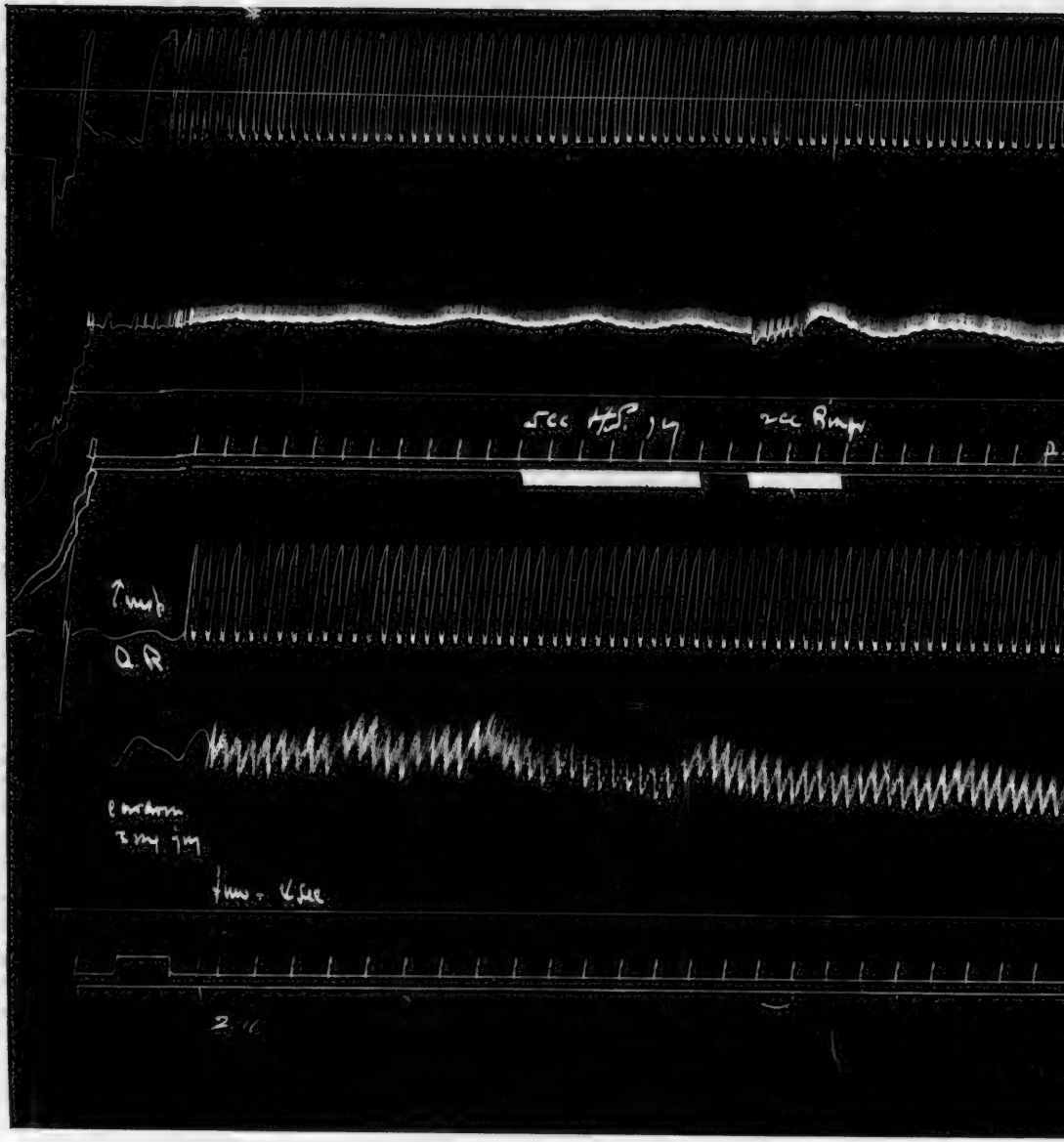
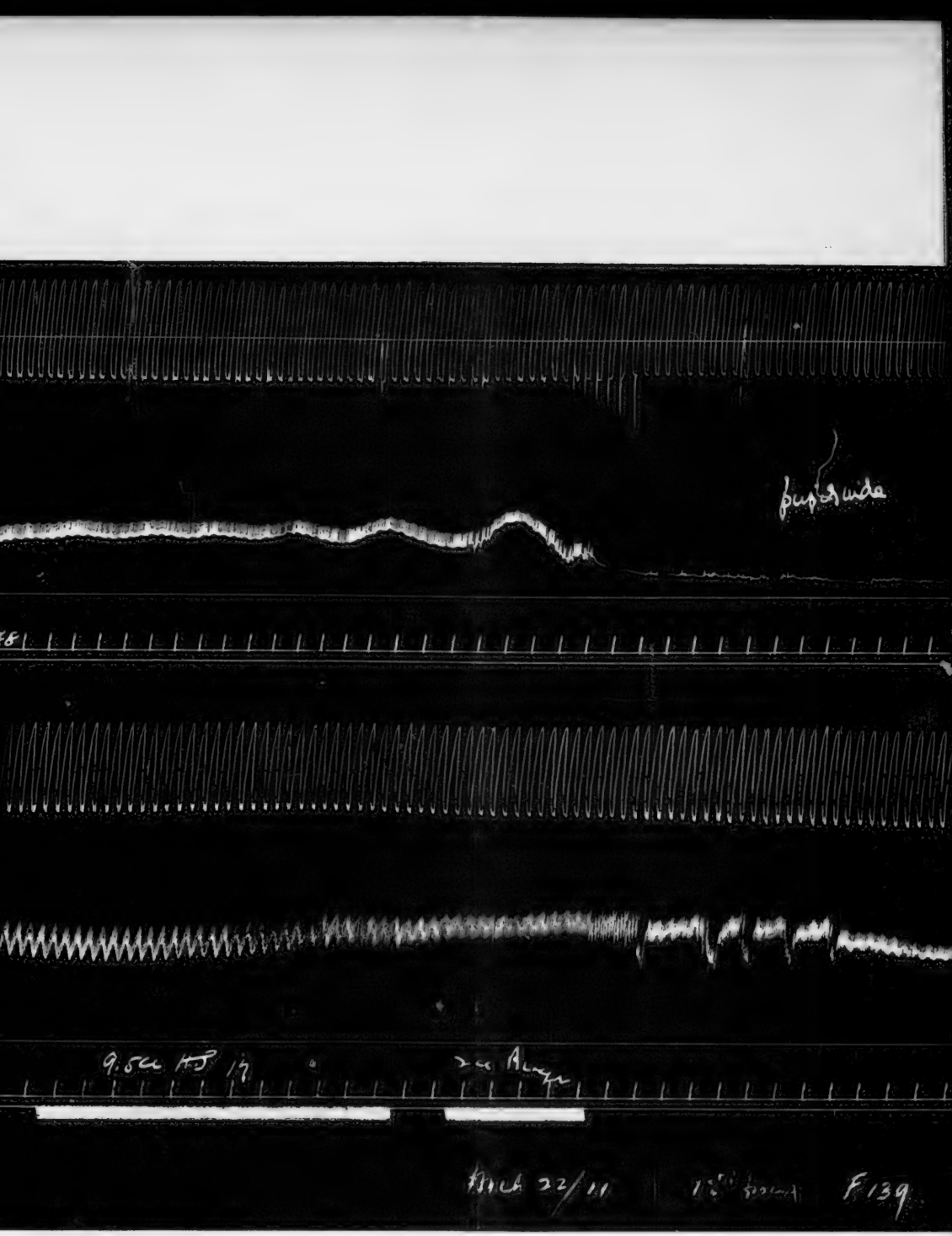
PLATE 45







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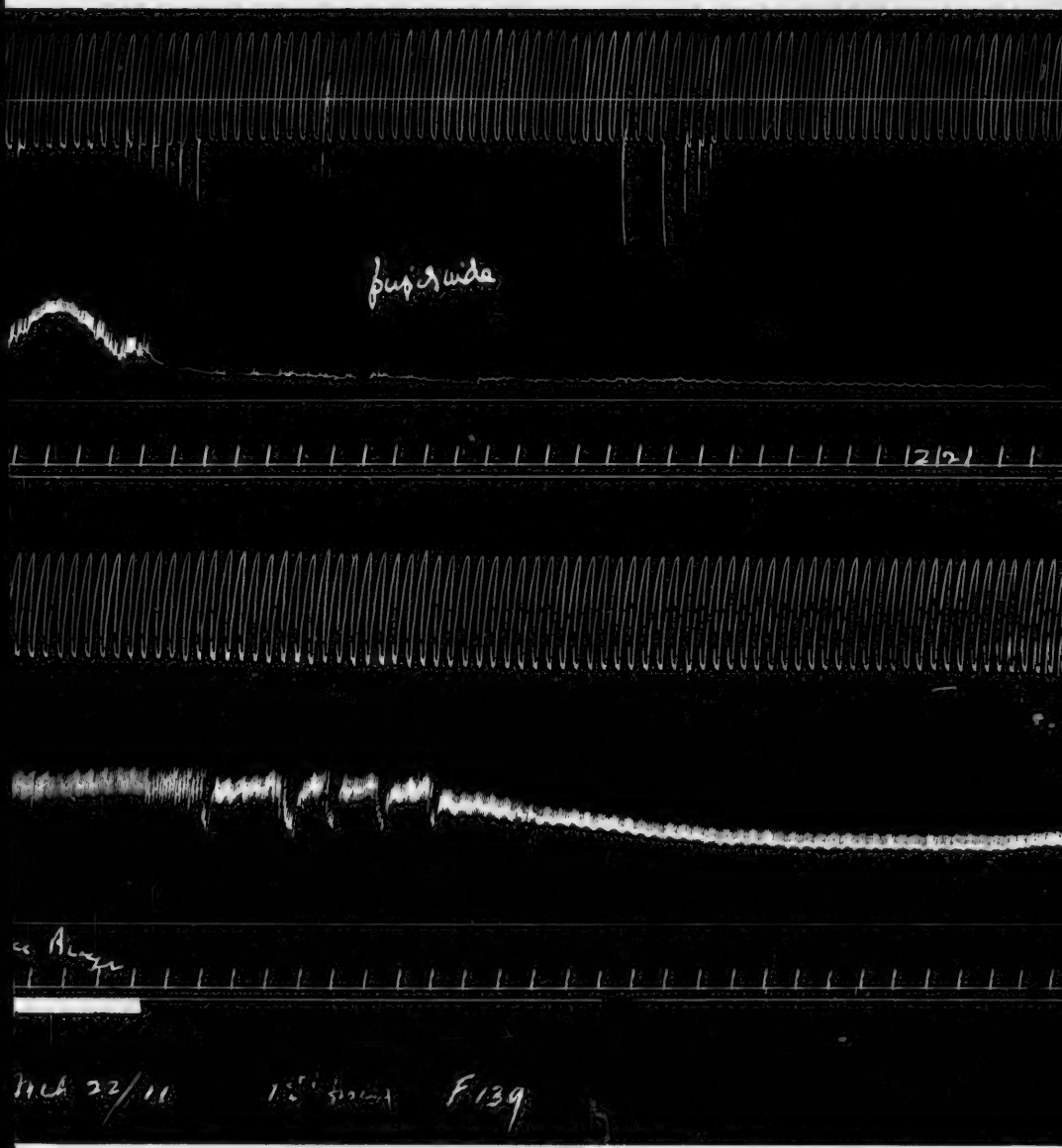
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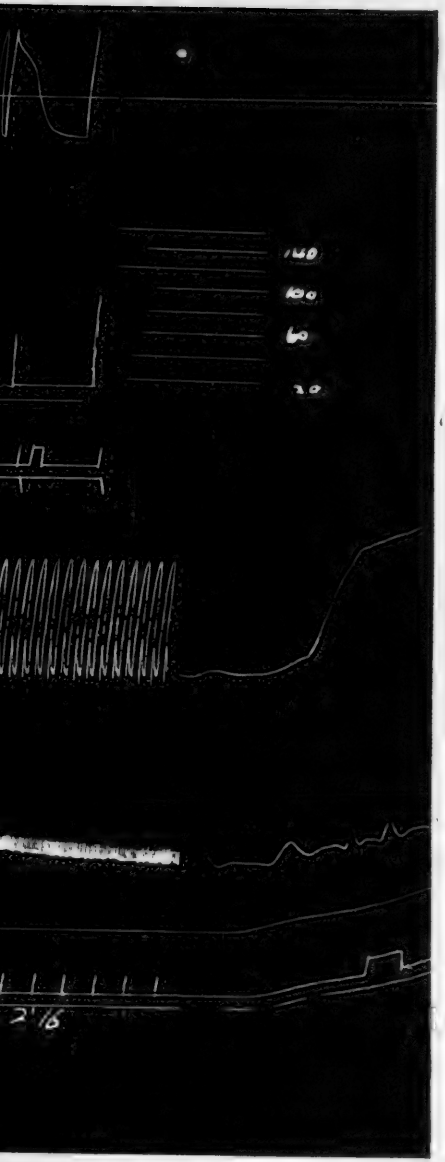
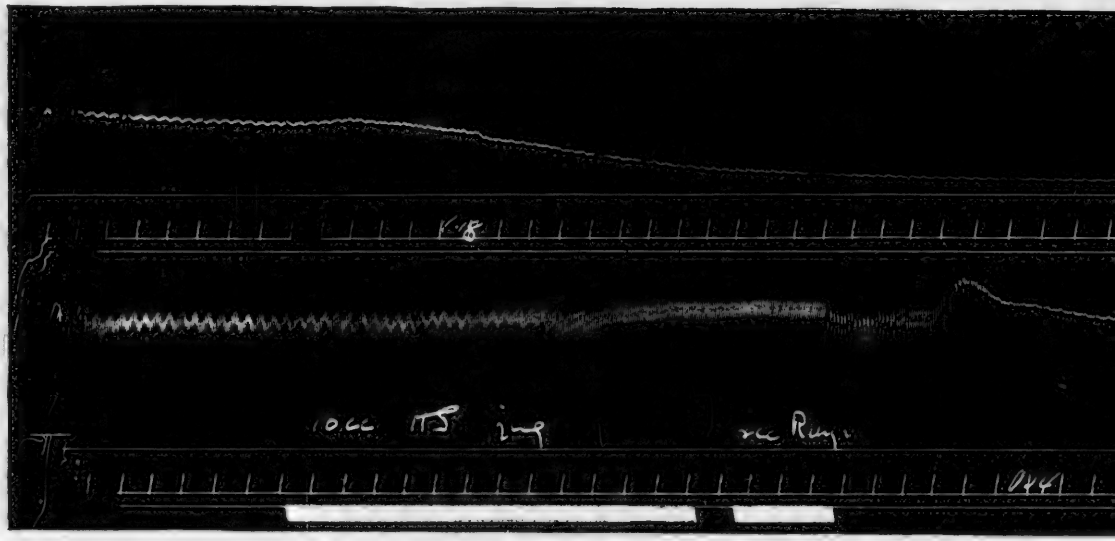
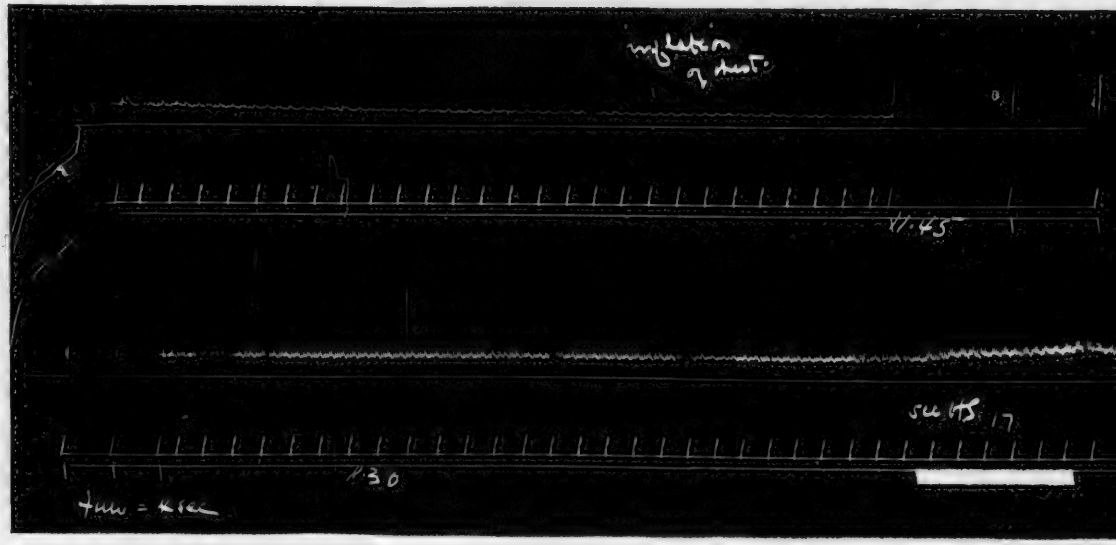
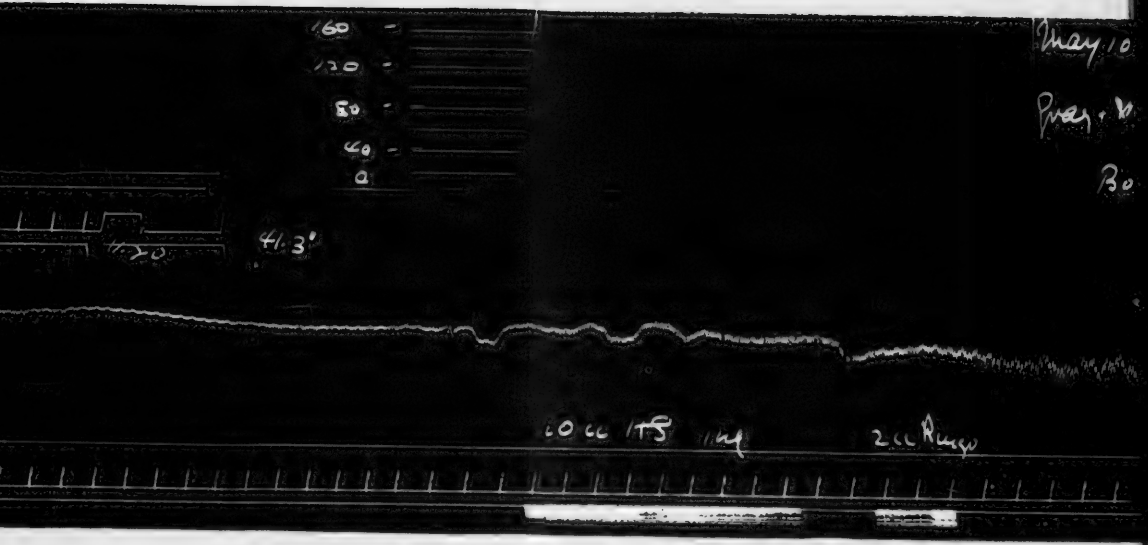
PLATE 46.









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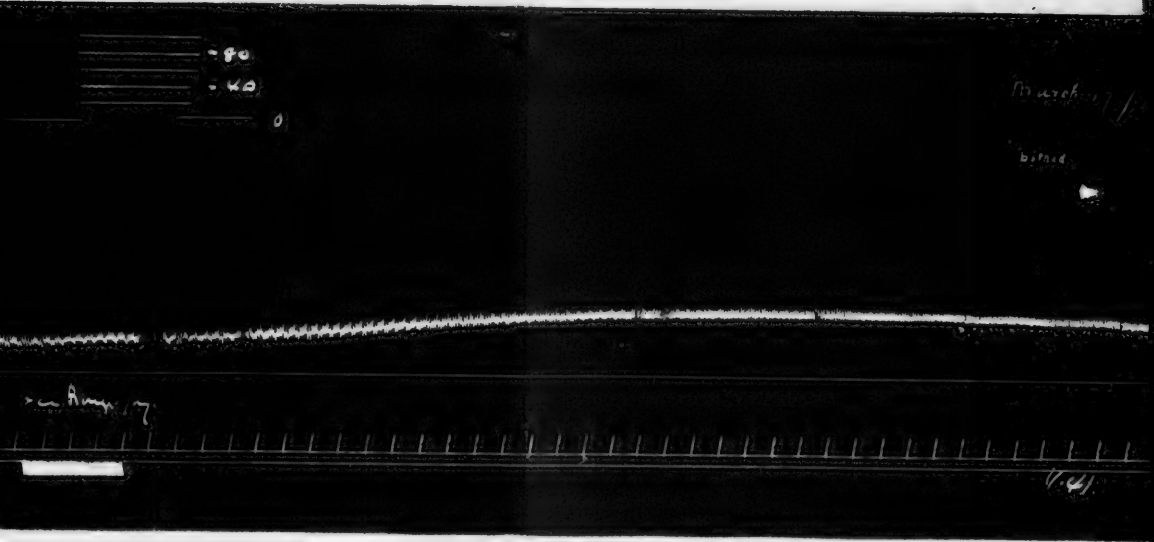

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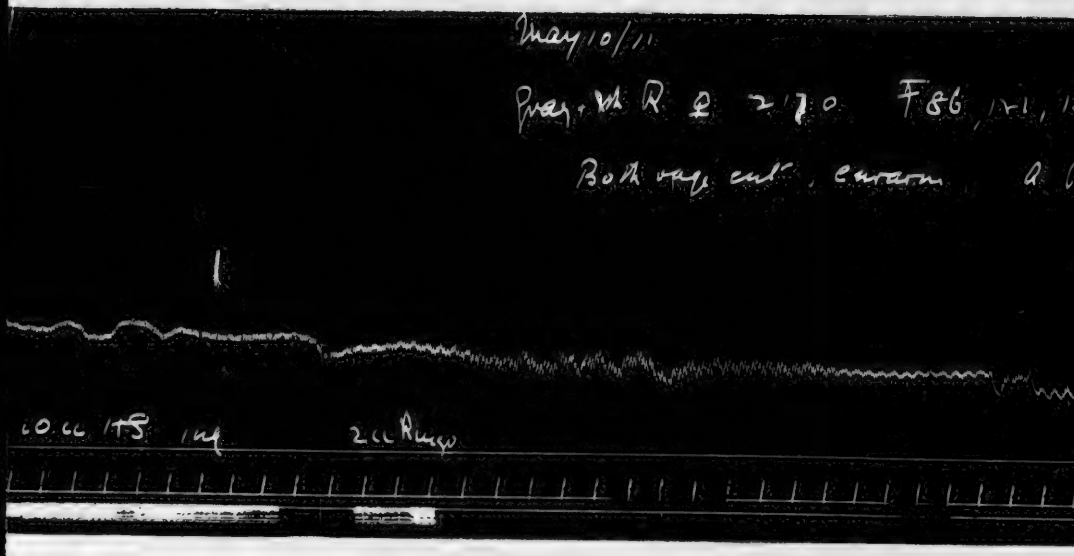
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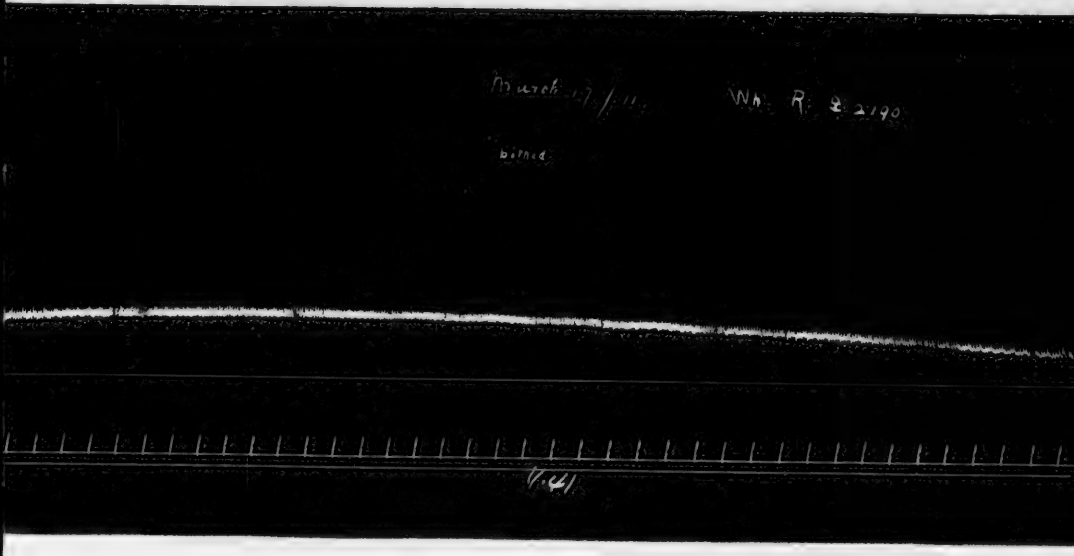
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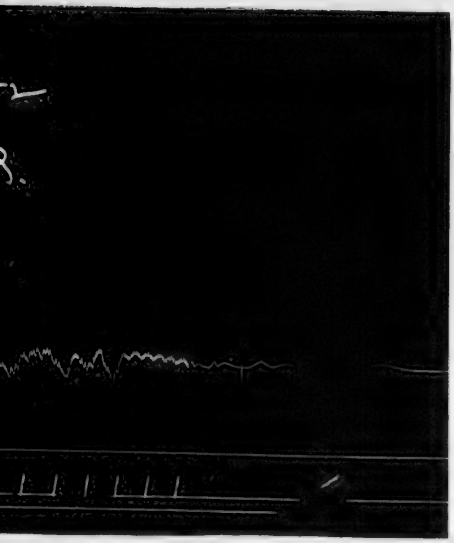
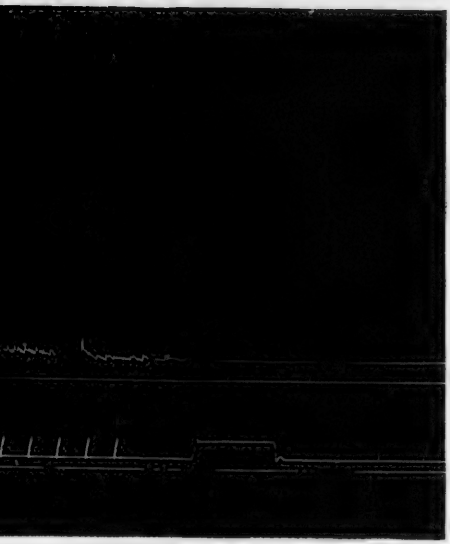
PLATE 47.





A NOTE ON SALVARSAN AND ACUTE ANAPHYLAXIS.*

By J. AUER.

(*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research, New York.*)

While testing the toxicity of acid 0.1 per cent. salvarsan solution when injected intravenously in rabbits,[1] it was noticed that the animals stood a second injection after an interval of two to four weeks just as well as the first one. This was of some interest because, if the drug sensitized the animals, that is, rendered them anaphylactic, this second injection ought to have produced symptoms characteristic of this interesting condition. In order to test still further whether or not salvarsan can produce a state of hypersensitiveness, a number of guinea pigs also were used, for these animals can be sensitized more readily and more certainly than rabbits, at least as far as horse serum is concerned.

It must be emphasized at once, however, that the main object of this investigation was not to establish whether anaphylaxis of any degree could be obtained with salvarsan, but only to find out whether a second or third injection could produce dangerous and fatal consequences.

Both acid and alkaline solutions were used, and the strength was chiefly 0.1 per cent. The alkaline solution was prepared by adding one cubic centimeter of normal sodium hydrate for every 0.1 gram of the drug which had been ground into solution with a small amount of 0.9 per cent. saline. This solution was then further diluted by 0.9 per cent. saline.

As criteria for the state of acute anaphylaxis, the clinical signs and pathological picture which Lewis and I[2] have proven to be characteristic for guinea pigs were to be used; for rabbits, the train of symptoms and physiological findings which I have de-

*Received for publication, August 21, 1911.

[1] Auer, *Arch. Int. Med.*, 1911, viii, 169.

[2] Auer and Lewis, *Jour. Am. Med. Assn.*, 1909, liii, 458; *Jour. Exper. Med.*, 1910, xii, 154–157.

scribed some time ago[3] were similarly to be employed. It was not necessary, however, to use them, for both species of animals showed no definite response to the second or toxic injection.

RESULTS.

Guinea Pigs.—The average weight of the ten guinea pigs used was 500 to 600 grams. The first injection of the drug was given either intraperitoneally or into the jugular vein. The amount injected was always either five or ten milligrams in 0.1 per cent. acid solution (*viz.*, dissolved merely in 0.9 per cent. saline). The animals stood the injection of the rather large amount well, and showed no marked toxic symptoms. All of them were, however, very quiet for hours afterwards, and did not run about, but crowded together in a corner of the cage. The next day, as a rule, they were lively. The intraperitoneal injection seemed to cause more discomfort than the intravenous one.

The second injection was given after a time which varied between 17 and 127 days and was always intravenous (jugular vein). This second injection was usually an alkaline 0.75 per cent. solution; occasionally 0.1 per cent. acid solution was employed. The amount injected for the second dose was always ten cubic centimeters.

The animals stood the second injection well, and not a single fatality occurred. A few showed a moderate sinking in of the chest during inspiration, some minutes after the injection, which could be interpreted as a sign of some respiratory stenosis;[4] these symptoms were, however, never extreme. On killing an animal, during this stage, the lungs failed to show any indication of the characteristic inspiratory immobilization which Lewis and I have described[5] for acute horse serum anaphylaxis in guinea pigs.

Most of the guinea pigs lost weight after an injection of salvarsan, but this was more pronounced and more lasting after the second injection than after the first. After the second injection it usually took three to four weeks before the weight at the time of reinjection was again regained.

[3] Auer, *Zentralbl. f. Physiol.*, 1910–11, xxiv, 957.

[4] See the description of horse serum anaphylaxis in guinea pigs, Auer and Lewis, *Jour. Exper. Med.*, 1910, xii, 154–155.

[5] Auer and Lewis, *ibid.*, 156.

Rabbits.—Eight rabbits received at least two injections of salvarsan. The first injection was given either through the ear vein or into the jugular vein, in 0.1 per cent. acid solution; in one case only was 0.5 per cent. employed. The initial dose varied between seven and twenty-two milligrams per kilo. The second injection was given sixteen to twenty-nine days later, except in one instance where six months were allowed to elapse. The dose for the second injection varied between twelve and twenty milligrams per kilo; here again the acid 0.1 per cent. solution was utilized. The second injection was borne as well as the first, and there were no prostrations or sudden deaths. Four of these rabbits, which had already been injected twice, were reinjected a third time, about five months after the second injection. For the third injection an alkaline solution of 0.75 per cent.[6] was employed. The dose injected was fifteen milligrams per kilo, and it was incorporated through a lateral ear vein.

The injection was endured without a struggle. For some time afterwards the animals were quiet, but showed no definite signs of weakness or prostration. No feces or urine were passed during the two subsequent hours that the animals were under observation. There were no sudden deaths.

All of these animals lost weight moderately after the injection. Two of them regained their normal weight within a week; the two others had not fully recovered their initial weight after one month. It must be added that the greatest loss recorded did not exceed 150 grams. All of these rabbits seemed to be in excellent condition shortly afterwards, and are still alive and well one month after the third injection.

From the brief description given above, it is clearly shown that spaced injections of 0.1 per cent. of salvarsan, the time interval being two weeks, four weeks, and five months, cause no acute anaphylaxis in guinea pigs and rabbits.

It is also shown that repeated spaced injections of fairly large doses of salvarsan in 0.1 per cent. strength cause no cumulative effects in rabbits.

[6] This strength was used through error; a 0.3 gram capsule of salvarsan was taken for making the solution, instead of a 0.4 gram container.

ON HYPERTROPHY AND REGENERATION OF THE ISLANDS OF LANGERHANS.*

By RUSSELL L. CECIL, M.D.

(*From the Pathological Laboratory of the Presbyterian Hospital, New York.*)

PLATES 48 AND 49.

At the present time, there are two crucial problems in connection with the islands of Langerhans which must be solved before the "island theory" can be firmly established. The first, a morphological one, concerns the origin of the islands and the relationship existing between them and the glandular acini; the second, a physiological one, has to do with the connection between the islands and carbohydrate metabolism. It is of certain phases of the former problem that I propose to treat in this paper.

DEVELOPMENT OF THE ISLANDS OF LANGERHANS IN THE FETAL PANCREAS.

Before taking up the subject of hypertrophy and regeneration of the islands, it will not be amiss to review briefly the mode of development of the islands in the fetal pancreas. In this field of research on the islands, the results have been fairly harmonious. Laguesse (1), studying the histogenesis of the islands in sheep embryos, has shown that the islands are of epithelial origin, and develop in common with the acini from the primary tubules. Pearce (2), Küster (3), and Weichselbaum and Kyrle (4) have all studied the development of islands in the human embryo, and have reached almost identical conclusions. The results of their investigations are well summarized in the closing paragraphs of Küster's paper:

(1) The islands of Langerhans appear in early embryonic life as anatomically differentiated structures in the pancreas.

(2) The earliest anlage is found in offshoots from the ducts.

*Received for publication, August 5, 1911.

(3) The anlage is marked by three characteristics:
  (a) The nuclei are centrally located.
  (b) The cells arrange themselves in bands or rows.
  (c) The cells are in close contact with capillaries.

THE ISLANDS OF LANGERHANS IN CONGENITAL SYPHILIS OF THE PANCREAS.

In congenital syphilis of the pancreas, the normal development of the gland is retarded so that the various structures bear more or less resemblance to those observed in fetal life. Opie (5), Pearce, and more recently Karakascheff (6), have studied the islands in syphilitic pancreatitis and have noted that the islands, instead of being independent of other structures, are in many instances connected with the ducts or acini by slender cords or tubules of cells.

I have had the opportunity of studying sections from three cases of congenital syphilis of the pancreas.

Case 1.—(Dr. Opie's case.) Infant, 40 cm. in length; lived three hours. *Anatomical diagnosis.*—Congenital syphilis, interstitial pneumonia, splenic tumor, chronic perisplenitis. *Pancreas.*—Microscopically, the interstitial tissue is greatly increased. The lobules are small and widely separated. The islands are imbedded in the stroma, but have not been invaded by the fibrous tissue. Some of them are in direct connection with the ducts by means of slender tubules lined with flattened epithelial cells (figure 1).

Case 2.—(Dr. Opie's case.) Infant, 50 cm. in length; lived four hours. *Anatomical diagnosis.*—Congenital syphilis, pemphigus neonatorum, interstitial pneumonia, interstitial hepatitis and pancreatitis, splenic tumor. *Pancreas.*—Microscopically, the interstitial tissue is greatly increased. The lobules contain fewer acini than normally. The islands are numerous and surrounded by dense bands of fibrous tissue. Some of the islands are in continuity with ducts, as in case 1.

Case 3.—(Presbyterian Hospital, autopsy No. 8,050.) Infant, male, 18 months old. *Anatomical diagnosis.*—Congenital syphilis, interstitial hepatitis, nephritis, pancreatitis, acute broncho-pneumonia. *Pancreas.*—Microscopically, there is a well marked increase of the interstitial tissue. The lobules are uneven in size, but the acini appear normal. The islands are numerous and well preserved. A few of them are found to be connected with ducts by short, slender tubules lined with flattened epithelial cells.

In the protocols of these cases, it will be observed that two of them were new-born, while the third was an infant eighteen months old. In all three cases, there is a well marked interstitial pan-

creatitis, with more or less replacement of the parenchyma by fibrous tissue. The islands of Langerhans, however, are well preserved and quite numerous. Some of the islands are independent of the surrounding tissue, while others are in direct continuity with the ducts or acini. Occasionally one encounters a small duct from which islands and acini can be seen budding side by side. The connecting link between the island and the duct is a slender cord or tubule composed of a single or double row of low cuboidal epithelial cells (figure 1).

A careful study of the sections from these cases will convince one that Pearce and Opie are correct in considering the changes described as the result of interference with normal growth. The islands of Langerhans, instead of separating from the ducts, as they do under normal conditions, often remain in continuity with the tubular structures from which they have originated. The histological picture is that of a fetal pancreas, the seat of chronic interstitial inflammation.

HYPERTROPHY AND REGENERATION OF THE ISLANDS OF LANGERHANS.

In a previous paper, I (7) have emphasized the fact that hypertrophy of the islands may occur in one or the other of two forms. In the first, or simple hypertrophy, the island may show no abnormality except an increase in size, the arrangement of the cells in irregular cords being still preserved. In the second or columnar type of hypertrophy, the islands assume certain abnormal characteristics, the most striking of which is a change in the shape of the cells from a polyhedral to a high cylindrical form, with centrally placed nuclei.

Regeneration or new formation of the islands from the ducts was first described by Weichselbaum (8) in 1908. In 19 out of 151 cases of diabetes, he noted small buds composed of high cylindrical cells, which were growing out from the ducts, and which in serial sections he could demonstrate as connecting links between the ducts and rudimentary islands. These rudimentary islands were also composed of high columnar cells. As the newly formed

islands grew larger, they became separated in some instances from the ducts; but a duct could usually be found in the neighborhood of such islands. Weichselbaum thought this mode of regeneration of islands corresponded to that of their embryonic development as described by Pearce, Küster, and Karakascheff.

In the same year that Weichselbaum reported these observations, his pupil, Kyrle (9), described similar regenerative processes in partially depancreatized dogs and guinea pigs. In the neighborhood of the scars, he found many mitotic figures in the cells lining the pancreatic ducts and small buds which differentiated into new acini or new islands. He observed many giant islands but considered the regenerative processes more important from a compensatory standpoint than the hypertrophy of already existing islands.

The researches of Weichselbaum and Kyrle have explained, therefore, in a very satisfactory way the occurrence of two types of island hypertrophy. Simple hypertrophy is merely an enlargement of a preëxisting island. Columnar hypertrophy, on the other hand, is seen in connection with islands newly formed from the ducts.

During a previous study of the islands of Langerhans in diabetes, I (7) became interested in the subject of hypertrophy of the islands; and having since encountered the condition in a number of cases where there was no evidence of diabetes, it seemed worth while to review all the material at hand and, if possible, to contribute more knowledge to the subject. Such a study now appears the more desirable since Herxheimer (10), in his recent investigations on the islands after ligation of the pancreatic duct, has been unable to find any islands budding from ducts as described by Weichselbaum and Kyrle.

The cases in which I have encountered columnar hypertrophy and regeneration of the islands of Langerhans may be divided into the following groups: (1) diabetes mellitus; (2) chronic pancreatitis; (3) carcinoma of the pancreas; (4) cases in which the pancreas is for the most part normal.

In some cases, the hypertrophy has been more marked than the regenerative changes; in others, the reverse has been true. The

hypertrophy is of two types: (1) Simple hypertrophy, in which there is more or less increase of size in preëxisting islands. Such islands preserve their normal round or oval contour and their characteristic architecture. I have never seen islands of this type exceed a diameter of 0.6 of a millimeter. (2) The so-called columnar hypertrophy. This type is much more common. Here the structure of the island is changed, the cells being of a high cylindrical form and arranged in rows like columnar epithelium. In shape, the cells bear considerable resemblance to those lining the larger ducts. The cytoplasm takes the same bright stain with eosin and appears homogeneous. The nuclei, however, unlike those in the cells of the ducts are centrally located. They are large and vesicular and contain several chromatin granules. The cells are arranged side by side in curving and anastomosing bands or rows, sometimes singly, sometimes several rows together. The intimate relation of cells to capillaries is analogous to that seen in the normal island. The cords of cells twist themselves into various shapes, sometimes forming loops that simulate acini; but the absence of zymogen granules and the presence in many instances of a capillary in the lumen of such structures show their real nature. One of the striking traits of these columnar islands is their irregular contour, which is produced by the outgrowth of loops and processes of columnar cells into the surrounding tissue.

Hypertrophied islands of the columnar type may reach dimensions many times greater than those of normal islands. I (7) have recently reported one such island that measured 4 by 3.5 millimeters, and a number of others as large or larger have been reported. Diameters of 0.5 to 1.0 millimeter are not unusual in these cases.

Columnar islands are usually found in the neighborhood of ducts. In serial sections, a direct connection with the duct can often be demonstrated, in which case the island may be either sessile, or connected with the duct epithelium by a slender cord of cylindrical cells (figures 2 and 3).

Regeneration or new formation of islands I have usually found associated with columnar hypertrophy, for the obvious reason that they represent different stages of one and the same process. A

small bud or knob of cylindrical cells grows out from the duct, and, as the cells multiply, assumes the form of a small island of the columnar-cell type. The process corresponds closely to that described in connection with the development of the islands in the fetal pancreas. The newly formed islands exhibit a capacity for growth much greater than that of preëxisting islands. One may, therefore, encounter all sizes of columnar islands from that of a bud consisting of only a few cells to that of one of the giant islands already referred to. Moreover, these large islands may send out an offshoot of cylindrical cells several hundred micromillimeters in length, at the end of which another columnar island will develop. Working as I have with autopsy material, I have naturally seen very few mitotic figures in either the ducts or the newly forming islands; but that they occur in large numbers in freshly fixed tissue, as described by Weichselbaum and Kyrle, I do not doubt. The islands develop from the small or moderate sized ducts. I have seen large islands connected with very small ducts and small islands connected with fairly large ones.

The relation which these hypertrophied and newly formed islands bear to the acini is an important phase of the subject. Is it possible for new islands to develop from acini? Can new acini develop from columnar islands? Kyrle, in his experiments on animals, found that new acini as well as new islands could develop from the ducts, but he was never convinced that either one of them might develop from the other. There are undoubtedly columnar islands that are connected neither with the ducts nor with other islands; and there are columnar islands whose columns of cells are apparently continued into glandular acini. It is very hard to decide, however, whether this connection of islands with acini is real or apparent. My own observations have led me to favor the latter view. Newly forming islands, pushing their way in between acini, come into such intimate contact with acinar cells that in microscopical sections one might readily be deceived by the picture. If there is an anatomical connection between the two, I should be inclined to favor the view that new islands were being differentiated from acini, such a process being more in harmony with the fetal

development of the islands than a development of acini from islands.

HYPERTROPHY AND REGENERATION OF THE ISLANDS OF LANGERHANS IN DIABETES.

Since Opie's study of the islands of Langerhans in diabetes, considerable attention has been given to the degenerative and fibrous changes in the islands so frequently encountered in this disease. Comparatively little, however, has been written about hypertrophy and regeneration of the islands in diabetes. Since lesions of the islands are generally found in association with diabetes, it would be in connection with diabetes that one would expect to find regenerative hypertrophy of the islands most frequently; and such indeed is the case. Herxheimer (11) was one of the first to observe hypertrophy of the islands. He describes some very large islands which he encountered in two cases of diabetes. Hypertrophied islands in association with diabetes have also been observed by Reitmann (12), Ssobolew (13), MacCallum (14), Karakascheff (6), and me (7).

These investigators all noted the peculiar columnar arrangement of the island cells in such cases, but offered no explanation of it. Weichselbaum, in the study already referred to, found that the columnar islands were always in the neighborhood of ducts and in some instances directly connected with them.

I have studied the pancreas in one hundred cases of diabetes, with special reference to hypertrophy and regeneration of the islands. In thirty-four out of the hundred cases, I have found one or the other condition present; usually both. By referring to the table, it will be seen that the lesions occurred at all ages and that, although they were generally found in association with chronic interacinar pancreatitis, there were eight cases where there was no interstitial new growth and eleven others in which the fibrosis was inconsiderable.

Other lesions of the islands were associated with hypertrophy and regeneration in thirty-one out of the thirty-four cases. The lesions found were hyaline degeneration in eight cases, round cell infiltration in two, and sclerosis in twenty-eight cases. The three

CASES OF DIABETES SHOWING HYPERTROPHY AND REGENERATION OF THE ISLANDS.

Case number.	Age in years.	Pancreas.	Islands of Langerhans.
Case 1. Presbyterian Hospital.	57	Well marked chronic interstitial pancreatitis.	Sclerosis and hyaline degeneration. Considerable hypertrophy, sometimes columnar.
Case 2. Presbyterian Hospital.	27	Normal.	Small and very scanty. A few small columnar-celled islands.
Case 3. Presbyterian Hospital.	35	Moderate grade of chronic interstitial pancreatitis.	Quite scanty. Moderate sclerosis. A few small columnar-celled islands.
Case 4. Presbyterian Hospital.	60	Well marked chronic interstitial pancreatitis.	Considerable sclerosis. Many hypertrophied islands of the columnar type.
Case 5. Presbyterian Hospital.	52	Considerable chronic interstitial pancreatitis.	Scanty and show considerable sclerosis. A few small columnar-celled islands.
Case 6. Presbyterian Hospital.	75	Considerable chronic interstitial pancreatitis and fatty infiltration.	Sclerosis and hyaline degeneration. Many hypertrophied islands of columnar type. Some apparently connected with ducts.
Case 7. Dr. James's case.	36	Advanced chronic interstitial pancreatitis.	Considerable sclerosis and hypertrophy of islands, a few of them of the columnar-cell type.
Case 8. Royal Victoria Hospital.	20	Simple atrophy of pancreas. Microscopically no increase of interstitial tissue.	Infiltration of leucocytes about some islands. Considerable hypertrophy. A few small columnar islands.
Case 9. Royal Victoria Hospital.	31	Moderate grade of chronic interstitial pancreatitis.	Marked hypertrophy of columnar type and extensive new formation of islands from ducts and acini. Island capillaries sclerotic.
Case 10. Royal Victoria Hospital.	75	Well marked chronic interstitial pancreatitis.	Considerable sclerosis and hypertrophy. One giant island of columnar type, 1,800 micromillimeters in diameter.
Case 11. Royal Victoria Hospital.	29	Considerable interstitial new growth, and infiltration of fat.	Considerable sclerosis and hypertrophy. A few columnar-celled islands, one of them in direct connection with a duct.
Case 12. Royal Victoria Hospital.	70	Well marked chronic interstitial pancreatitis.	Marked hypertrophy and regeneration of islands from ducts and acini, almost entirely of the columnar type. Slight sclerosis.
Case 13. Royal Victoria Hospital.	38	Slight increase of interstitial tissue in places.	Moderate sclerosis. Infiltration of lymphoid cells about someislands. Marked hypertrophy (columnar) and regeneration from ducts.
Case 14. Montreal General Hospital.	28	No interstitial new growth, but infiltration of leucocytes about vessels.	Slight sclerosis, considerable hypertrophy. A few small columnar-celled islands.
Case 15. Montreal General Hospital.	66	Slight amount of chronic interstitial pancreatitis and considerable infiltration of leucocytes.	Well marked sclerosis and hypertrophy. Many hyaline islands. A few columnar islands about ducts.

Case number	Age in years.	Pancreas.	Islands of Langerhans.
Case 16. Montreal General Hospital.	26	Considerable infiltration of leucocytes.	Infiltration with leucocytes and considerably hypertrophied. A few islands of the columnar type.
Case 17. Mass. General Hospital.	45	Advanced sclerosis of pancreas.	Islands small, scarce, sclerotic. A few of them are columnar in type.
Case 18. Mass. General Hospital.	58	Moderate interstitial pancreatitis.	Sclerosis and hyaline degeneration. A few large columnar islands near ducts.
Case 19. Mass. General Hospital.	53	Mild grade of chronic interstitial pancreatitis.	Capillary walls thickened. Considerable number of large islands of the columnar type near ducts.
Case 20. Mass. General Hospital.	70	No changes except arteriosclerosis.	Moderate sclerosis. A few small columnar-celled islands.
Case 21. Mass. General Hospital.	20	Normal.	Small and scarce. A moderate number of small columnar-celled islands.
Case 22. Mass. General Hospital.	60	Advanced interstitial pancreatitis.	Considerable sclerosis. Number of islands small. A few small columnar islands.
Case 23. Mass. General Hospital.	59	Well marked chronic interstitial pancreatitis.	Small and scarce. Moderately sclerotic. Many large columnar islands, some connected with ducts.
Case 24. Mass. General Hospital.	31	Well marked chronic interstitial pancreatitis.	Scarce. Well marked sclerosis. A few small columnar islands.
Case 25. Boston City Hospital.	27	Normal.	Small and scarce but free from sclerosis. A few small columnar islands, apparently connected with acini.
Case 26. Boston City Hospital.	59	Moderate grade of chronic interstitial pancreatitis.	Sclerosis and hypertrophy. Some show hyaline changes. A few columnar islands, one connected with a duct.
Case 27. Pennsylvania Hospital.	51	Well marked chronic interstitial pancreatitis.	Scarce; moderately sclerotic. Some of the islands of the columnar type. One of them connected with a duct; one with acinus (?).
Case 28. New York City Hospital.	50	Marked fatty infiltration.	Numerous; sclerotic, hyaline, hypertrophied. A few large columnar islands.
Case 29. Bellevue Hospital.	33	Normal.	Sclerotic, some hyaline, marked hypertrophy of the columnar type.
Case 30. Bellevue Hospital.	73	Moderate grade of chronic interstitial pancreatitis.	Considerable sclerosis and hyaline degeneration. A good many hypertrophied islands of the columnar type, one connected with duct.
Case 31. Mt. Sinai Hospital.	73	Moderate grade of chronic interstitial pancreatitis.	Scarce; sclerotic and hyaline. Many hypertrophied columnar islands about ducts.
Case 32. Presbyterian Hospital.	52	Mild chronic interstitial pancreatitis.	Marked hyaline degeneration of nearly all islands. A few large columnar islands about ducts, one in direct connection.

Case number.	Age in years.	Pancreas.	Islands of Langerhans.
Case 33. Presbyterian Hospital.	57	Mild chronic interstitial pancreatitis.	Large number of columnar islands, some very large; some connected with ducts (others with acini?). Moderate sclerosis.
Case 34. Bellevue Hospital.	60	Well marked chronic interstitial pancreatitis.	Sclerotic and hyaline. Remarkable hypertrophy of islands in head of pancreas. All of columnar type and connected with ducts or acini.

cases in which no other changes were found occurred in young people; and in all three instances the islands were noted as small and scanty.

In twelve of the thirty-four cases, columnar islands were present, but no hypertrophied forms were encountered. Larger forms would doubtless have been found in some of the cases if more sections had been accessible. On the other hand, I have seen several specimens in which a simple hypertrophy of the islands existed, but where the columnar islands were lacking; that is, the preëxisting islands had increased in size, but there had been no new formation of island tissue.

There was no apparent relation between the severity of the lesions in the islands and the extent of hypertrophy and regeneration of islands. For example, in case 32, practically every preëxisting island in the section had been converted into hyaline material and only a few newly formed columnar islands had appeared. On the other hand, in case 9, there was only a slight sclerosis of the normal islands, and a remarkable hypertrophy and regeneration of the columnar type of island was observed.

HYPERTROPHY AND REGENERATION OF THE ISLANDS OF LANGERHANS IN CHRONIC PANCREATITIS.

Weichselbaum (4) has observed regeneration of the islands of Langerhans in some cases of chronic interstitial pancreatitis, where there was no diabetes. I have examined sections from thirty-three cases of chronic pancreatitis of the interlobular type. In ten cases the changes were moderate; in eighteen they were well marked; and in five cases there was advanced sclerosis with extensive destruction of parenchyma.

The cases were associated with the following conditions:

Cirrhosis of liver	13 cases.
Gall stones	9 cases.
Cardiac and renal disease	6 cases.
Chronic cholecystitis	1 case.
Ulcer of stomach	1 case.
Jaundice	1 case.
Fatty liver	1 case.
Septic peritonitis	1 case.

In these thirty-three cases, the islands were in many instances bunched together and surrounded by dense fibrous tissue, but in other respects they appeared normal. In only three cases was there any evidence of a regeneration of the islands. Two of these cases were among the group associated with cirrhosis of the liver; the other case was that of septic peritonitis. Each of the cases exemplified a different grade of cirrhosis.

A few small groups of columnar cells, arranged in the characteristic manner, occurred here and there in the sections, but the hypertrophied forms were absent. The process was so inconsiderable that I have omitted protocols of the cases.

The significant fact brought out by the study of this group is that extensive destruction of the pancreas may occur without there being any compensatory hypertrophy or regeneration of the islands. The islands are spared and no strain is placed upon the function of carbohydrate metabolism. The contrast between this group and the diabetic group is striking. In the latter, some of the most marked examples of regeneration and hypertrophy occurred in pancreases that showed little or no change.

HYPERTROPHY AND REGENERATION OF THE ISLANDS OF LANGERHANS IN CARCINOMA OF THE PANCREAS.

In carcinoma of the pancreas, the new growth replaces in great part the secreting parenchyma, but the islands of Langerhans show a tendency to persist in the midst of cancerous tissue, just as they survive in advanced cirrhosis of the pancreas. That the islands, too, in some instances are finally destroyed is shown by the fact that in a certain number of cases of pancreatic carcinoma, sugar

is present in the urine, while in other cases an alimentary glycosuria is observed.

Pearce (15), in a study of carcinoma of the pancreas, noted a hypertrophy of the islands along the advancing edge of the tumor. Weichselbaum (8) has observed hypertrophy and regeneration of the islands in some cases of pancreatic carcinoma.

I have studied microscopical sections from seventeen cases of cancer of the pancreas, three of them primary, fourteen metastatic, in none of which is there any record of glycosuria. I have studied these cases with special reference to the islands of Langerhans. In six of them there was only a moderate invasion of the gland by the new growth; in the other remaining eleven cases there was extensive cancerous involvement, with considerable replacement of the parenchyma by the neoplasm. In those cases where the tumor had occluded the pancreatic duct, there was more or less interstitial pancreatitis. The islands, as a rule, appeared normal. In the advanced cases, they were often found bunched together in a fibrous stroma, as in cirrhosis of the pancreas, but still preserving their normal appearance. In several cases the islands had undergone more or less simple hypertrophy, some of them measuring 400 or 500 micromillimeters in diameter. In only one out of the seventeen cases was I able to find the characteristic columnar hypertrophy and regenerative changes which have been already described.

The patient, a woman, aged 64 years, was operated upon for gall stones. At operation, a small, very hard gall-bladder containing stones was removed. Microscopical sections through the thickened wall showed an adenocarcinoma. The patient died a few days later.

Anatomical Diagnosis.—Cholecystectomy; metastatic carcinoma of liver, pancreas, and retroperitoneal lymph glands; malignant stenosis of the pancreatic duct; chronic interstitial pancreatitis; fat necroses; general arterial sclerosis; jaundice.

The pancreas is enlarged and very hard. On section, the lobules are found separated by translucent tissue. There are several fat necroses in the tail of the pancreas. The pancreatic duct is greatly dilated. There are stenoses at several points.

Microscopical sections through the head of the pancreas show a marked increase of the interstitial tissue separating the lobules.

The latter are compressed and atrophied. In a number of places there occur masses of large epithelial cells, in some places closely packed together, in others displaying a somewhat tubular arrangement. The islands of Langerhans are numerous and considerably larger than normally and many of them show typical columnar cell hypertrophy. The columnar islands are usually in the neighborhood of ducts.

HYPERTROPHY AND REGENERATION OF THE ISLANDS OF LANGERHANS IN CASES WHERE THE PANCREAS WAS FOR THE MOST PART NORMAL.

In diabetes, chronic pancreatitis, and carcinoma of the pancreas, the occurrence of hypertrophy and regeneration of the islands may be explained more or less satisfactorily. There remains, however, still another class of cases, a particularly interesting group, where, with none of these diseases present, the islands show definite hypertrophic and regenerative changes.

In the course of routine examinations of the pancreas from autopsies at the Presbyterian Hospital, I have encountered four such cases, and one other has been added through the kindness of Dr. Humphreys of the German Hospital, New York. The following are brief protocols of the cases.

Case 1.—M. M., female, aged 53 years, well nourished, moderately alcoholic. No evidence of lues. Admitted with typical symptoms of chronic nephritis. Died in uremic coma.

Presbyterian Hospital, Autopsy No. 7,923. Anatomical Diagnosis.—Chronic interstitial nephritis; infantile right kidney; arterial sclerosis; hypertrophy of heart; fatty liver; calcified nodes in lungs; chronic fibrous pleuritis; myoma of uterus; chronic cervicitis; vaginal ulcers.

The pancreas is firm, and on section it appears normal. Microscopically, there is a moderate amount of adipose tissue between the lobules, but little if any increase of the interstitial tissue. Marked endarteritis. The islands are very numerous and larger than normally. A considerable number of them are composed of columnar cells. The columnar islands are generally found about the small ducts and some are found connected with the ducts. In some of the larger branches of the pancreatic duct, the epithelium is thrown up into papillomatous folds which appear almost to occlude the lumen of the duct. These folds consist of a delicate central stalk of fibrous tissue which is covered with a single layer of very tall columnar epithelial cells, larger than those lining a normal duct. Here and there are groups of dilated acini, whose cells are flattened and hyaline and whose lumina are filled with a pink-staining, structureless material.

Case 2.—F. M., male, aged 25 years. Student. Admitted for mastoiditis. Had undergone an operation for nephrectomy sometime previously (pyonephrosis). Mastoid cells found filled with pus. Patient died in uræmic coma after operation.

Presbyterian Hospital, Autopsy No. 7,777. Anatomical Diagnosis.—Pyonephrosis and chronic interstitial nephritis of right kidney; absence of left kidney; chronic cystitis; chronic pulmonary tuberculosis; tuberculosis of peribronchial lymph glands; acute bronchopneumonia; edema of lungs and brain.

The pancreas is pale and firm; ducts and vessels are normal. Microscopically, the pancreas is free from sclerosis. A few dilated acini lined with flattened hyaline cells occur. The islands are numerous and in some cases considerably larger than normally. A moderate number of the islands are of the columnar-celled type and situated near the ducts. The rest of the islands are normal. The walls of the arteries are not thickened. The main duct appears normal. In some of the larger branches, however, the lining epithelium is thrown up into papillomatous folds, similar to those described in case 1. Many of the cells on those folds have taken on the goblet form.

Case 3.—J. D., female, aged 34 years. Moderately alcoholic. No evidence of lues. Marked obesity. Operated on for pus tube. Died twelve days after operation, after running a high continuous temperature.

Presbyterian Hospital, Autopsy No. 3,817. Anatomical Diagnosis.—Salpingectomy on right side; cloudy swelling of viscera; obesity; operation wound.

The pancreas weighs 180 grams. It is large, pale, and of firm consistence. Microscopically, the pancreas shows no increase of interstitial tissue, but there are small collections of lymphoid cells about some of the vessels and ducts. The main duct is entirely devoid of epithelium, being lined with cellular fibrous tissue. The islands are very numerous and show extreme hypertrophy of the columnar type. The picture resembles that seen in several of the diabetic cases.

Case 4.—J. W., male, aged 63 years. Temperate habits. No evidence of lues. Admitted to the hospital with symptoms of intestinal carcinoma. Markedly emaciated. Mass in right lower quadrant. Died twelve days after admission.

Presbyterian Hospital, Autopsy No. 7,810.[1] *Anatomical Diagnosis.*—Adenocarcinoma of colon; chronic interstitial nephritis; arterial sclerosis; hypertrophy of prostate.

The pancreas is firm and pale. The ducts are normal. Microscopically, the pancreas shows no increase in the stroma. The acini are normal. The islands are quite numerous and in some instances normal. The greater number, however, are composed of columnar cells and are larger than normally. Some of them are in direct connection with smaller ducts, while others are apparently connected with acini. One large island measures 4 mm. in diameter and is hyaline. There is infiltration of polynuclear leucocytes and lymphoid cells into the walls of the larger ducts, which also show hyaline changes. In some of the larger ducts, the epithelium is of the tall columnar type, folds of which project into the lumen.

Case 5.—A. B., male, aged 64 years. Admitted to the German Hospital, New York, in a moribund condition and markedly dyspneic. *Clinical diagnosis.*—asthma, emphysema, and myocarditis.

[1] This case has been reported by me in the *Journal of Experimental Medicine*, 1911, xiii, 595.

German Hospital, Autopsy No. 367. Anatomical Diagnosis.—Acute bronchopneumonia; acute and chronic bronchitis (asthma); emphysema; renal calculi; hydronephrosis; compensatory hypertrophy of right kidney; chronic interstitial nephritis; cardiac hypertrophy; arterial sclerosis.

The pancreas is fairly firm. On section, the middle portion of the duct is found to be markedly dilated, but there is no obstruction at its duodenal end. In the middle of the pancreas, near the duct are several small foci 5 to 10 mm. in diameter, which contain a greyish white, soft, coherent material. They do not look like cysts, but rather like masses of some tissue which is very soft; otherwise the lobulation of the pancreas is normal. Microscopically, sections through the soft white areas show them to be small tumors enclosed by fibrous capsules and composed of anastomosing epithelial processes which resemble the epithelium of ducts. These papillary processes are lined with high cylindrical cells some of which are goblet cells. Here and there in the tumor are small collections of mucoid material, evidently the secretion of the cells. The tumor has apparently been produced by the marked proliferation of the epithelium lining one of the ducts. The fibrous capsule of the tumor (wall of duct?) is infiltrated with lymphoid cells. Adjacent to one of the tumors there are several greatly dilated ducts. The parenchyma about the ducts shows a well marked interlobular sclerosis. Another section shows normal ducts and normal secreting parenchyma. The islands of Langerhans, however, are very numerous and many of them show considerable hypertrophy of the columnar type. The newly formed islands are usually in the neighborhood of ducts, and a direct continuation between island and duct can be demonstrated at one point. The larger arteries show advanced endarteritis.

We have then five cases of well marked regeneration of the islands of Langerhans in which there was no evidence of diabetes and no destruction of the pancreatic parenchyma by cirrhosis or cancer. There is, of course, no way of excluding an alimentary glycosuria or transient diabetes in any of them. Briefly they were: (1) cardio-renal disease, (2) pyonephrosis, (3) pyosalpinx, (4) carcinoma of colon, (5) cardio-renal disease.

Of the five cases, the two that occurred in young people were associated with infections, while of the other three, two were cardio-nephritics and one was a case of carcinoma of the colon. There is nothing in the cause of death in these cases to explain the regenerative changes in the islands. So far as we know, there is no connection between any of these diseases and carbohydrate metabolism. There is no associated lesion in another organ, common to all five cases, that would serve to explain the condition of the islands, nor does the gross appearance of the pancreas in this group

throw light on the question. Microscopically, however, a comparison of the pancreases is rather instructive.

Case number.	Condition of ducts.
1.	Adenomatous proliferation of lining epithelium of ducts. Some of the acini are dilated and lined with flattened hyaline cells.
2.	Adenomatous proliferation of lining epithelium. Some acini are dilated and lined with flattened hyaline cells.
3.	Collections of lymphoid cells about some of the ducts. The main duct is entirely devoid of epithelium, being lined with cellular connective tissue.
4.	Adenomatous proliferation of lining epithelium of large ducts. Coats of large ducts are hyaline and infiltrated with polynuclear leucocytes and lymphoid cells.
5.	Marked adenomatous proliferation of epithelium lining large ducts, producing obstruction of the lumen and dilatation of distal portion of duct. Infiltration of lymphoid cells into walls of ducts.

Of the five cases, three showed definite chronic inflammatory changes in the ducts; in four cases there was a well marked adenomatous proliferation of the epithelium lining the large or medium sized ducts. One or both of these conditions was present in each of the five cases. This adenomatous hypertrophy of the duct epithelium is the same as that which has been described by Winternitz (16) in association with chronic pancreatitis. The epithelium is thrown up into papillary folds, the lumen being often completely filled with them. The cells are no longer cubical but are high cylindrical; they stain deeply with eosin and often assume the goblet form. The nuclei are usually vesicular.

Winternitz offers the suggestion that the new formation of duct epithelium might be an effort on the part of the pancreas to regenerate after chronic inflammatory processes, but he thinks it more probable that the change in the ducts and the fibrous overgrowth may both be due to a third process, often some form of duct obstruction. From a study of my own cases, I am convinced that the duct changes may be independent of chronic pancreatitis.

In only one of the three cases which I have reported was there any interstitial new growth, and in that the sclerosis was confined to the neighborhood of the dilated ducts and apparently secondary to the duct changes.

It is, of course, impossible to establish a causal relationship between either inflammatory or adenomatous changes in the ducts and the regeneration of islands which we have seen associated with them. Since the islands regenerate from the ducts, it would be natural to suspect some connection between adenomatous hypertrophy of the duct epithelium and regeneration or adenomatous hypertrophy of the islands. This suspicion is enhanced by the similarity which exists between the two processes. I agree with Winternitz, however, that both changes should probably be attributed to a third common factor. In all the cases of this group, the regenerative changes are much more marked than the hypertrophic changes. Indeed, the columnar islands are often quite small and duct connections are readily found. In the absence of any evidence of carbohydrate derangement, it is hard to look upon this process as a compensatory one. It would rather seem to be a reaction following a disturbance of some kind in the ducts. Obstruction of the ducts, as suggested by Winternitz, may be the etiological factor in some cases. In one of my cases (case 5), there was marked dilatation of the larger ducts. In the one case of carcinoma of the pancreas where columnar hypertrophy and regeneration of the islands were observed, there was obstruction and dilatation of the main duct. In this instance, however, the proliferation of the duct epithelium was not noted.

These studies have shown that regeneration of the islands of Langerhans may occur in various conditions, and that such regeneration takes place by means of a budding off of columnar cells from the ducts. I am not sure that there is ever a real connection between these newly forming islands and the adjacent acini, though in some cases, like case 4 in the last group, the association is so intimate that the possibility of a direct connection cannot be excluded. The fact that processes of columnar cells push out between the acini and that in many places they are in immediate contact with acini would explain the apparent continuations of the one into

the other that are sometimes seen. The absence of regeneration of the islands in cases of advanced cirrhosis and carcinoma of the pancreas where the islands have been spared, and the occurrence, on the other hand, of regeneration and hypertrophy of the islands in many cases of diabetes where there has been little or no destruction of parenchyma, speak strongly for the functional independence of the islands and for their relation to carbohydrate metabolism.

The occurrence of hypertrophy and regeneration of the islands in cases where there is no evidence of diabetes may be explained in several ways. When associated with cirrhosis or carcinoma of the pancreas, we may look upon it as an effort on the part of nature to replace destroyed islands. In the five cases where regeneration of the islands has been associated with neither diabetes nor destruction of pancreatic tissue, an adequate explanation of their occurrence has not been offered. They have been associated, however, in every instance with abnormalities in the ducts; in three cases with chronic inflammatory changes; in four cases with adenomatous proliferation of the duct epithelium.

CONCLUSIONS.

1. Hypertrophy of the islands of Langerhans occurs in two forms: (1) the simple type which is nothing more than an increase in size of preëxisting islands; and (2) the columnar type, in which the islands are composed of anastomosing columns of cylindrical cells.

2. Regeneration of the islands of Langerhans takes place by means of a budding off of columnar cells from the ducts. The process is analogous to the development of the islands in the fetal pancreas.

3. Newly formed islands of Langerhans are composed of cylindrical cells similar in all respects to those constituting hypertrophied islands of the columnar type. The two structures are identical, the latter being a later phase of the former.

4. Newly formed islands of Langerhans are capable of a greater hypertrophy than preëxisting islands. Both types of insular hypertrophy are usually of a compensatory character, the columnar, or regenerative type, being the more important.

5. Hypertrophy and regeneration of the islands of Langerhans occur most frequently in diabetes mellitus (34 per cent. of 100 cases studied). These changes are usually associated with sclerosis or hyaline degeneration of other islands.

6. Hypertrophy and regeneration of the islands of Langerhans are occasionally observed in cirrhosis and carcinoma of the pancreas. In some of the most advanced cases, however, the islands have been spared and neither hypertrophy nor regeneration are present.

7. Regeneration of the islands of Langerhans has been noted in five cases in which there was no evidence of diabetes and where the pancreas was for the most part normal. In four of these cases, columnar hypertrophy of the islands was also observed. All five cases were associated with abnormalities of the larger ducts—obstruction, chronic inflammation, and adenomatous proliferation of the lining epithelium.

8. The occurrence of hypertrophy and regeneration of the islands of Langerhans affords considerable evidence in favor of their anatomic and functional independence.

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FIG. 1.

FIG. 2.

FIG. 3.

EXPLANATION OF PLATES.

Plate 48.

Fig. 1. Congenital syphilis. Case 1. The drawing shows an island of Langerhans surrounded by connective tissue, and directly continuous with a small duct.

Fig. 2. Diabetes. Case 33. In the centre, a medium sized columnar island is seen in connection with a small duct.

Plate 49.

Fig. 3. Diabetes. Case 12. An hypertrophied island of the columnar type is directly connected with a small duct.

A STUDY OF THE PRESPHYGMIC PERIOD WITH THE MICROGRAPH.*

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A series of tracings is given in text-figure 1, taken with films moving at the rate of 17.2 inches per second, in order to measure the time intervals with more accuracy than was possible with the lower speed. This speed is nearly three times that of the films from which the records were made in a former paper.[1] At this higher speed, approximately one cardiac cycle only is recorded on the length of film used. The vertical lines in the figure are spaced one tenth of a second apart. The line CC near the center of the figure is selected as a reference line at the one second mark, and marks the time of the beginning of the upstroke of the carotid pulse. Two curves were taken simultaneously in each case, the carotid always being one of the two. The series was obtained from the same subject as the set in text-figure 3 of a former paper,[2] and the apex tracing No. 24, text-figure 1, is very similar to tracings Nos. 2 and 3 in the figure referred to.

THE B-WAVE-GROUP.

The B-wave-group is very clearly marked in each of the pairs of simultaneous tracings Nos. 24 and 22, apex and carotid. Short vertical lines are drawn at the maximum and minimum points of the wave, and it will be seen that there is a fair approximation to regular periodicity. The period of the waves in the carotid, taking the average of a number of curves, is 0.0175 seconds, a distance of 0.3 inches in the figure; it represents a frequency of 57.4 complete waves per second. The period of the apex B-wave-group is a little

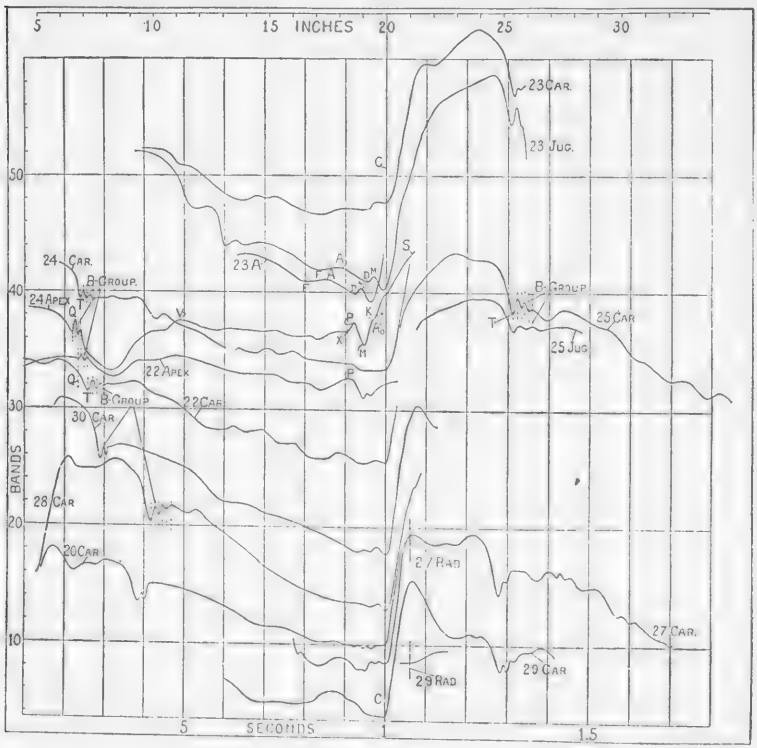
* Received for publication, July 31, 1911.

[1] *Jour. Exper. Med.*, 1911, xiv, 339.

[2] *Jour. Exper. Med.*, 1911, xiv, 354.

less, 0.014 seconds, a distance of 0.24 inches in the figure, and a frequency of seventy-one complete waves per second.

The first wave of the B-wave-group in the carotid pulse, No. 24, at the point T, occurs 0.33 inches or 0.0192 seconds later than the corresponding first wave at Q of the B-group in the simultaneous



TEXT-FIG. 1.

apex tracing No. 24. The pair of curves, No. 22, carotid and apex, gives 0.41 inches, or 0.0238 seconds, for the same time interval, and the average of the two sets gives 0.37 inches or 0.0215 seconds as the difference in time of the B-wave-group in the carotid and apex curves.

If we assume that the B-wave-group in both the apex and the carotid tracings is caused by the closing of the semilunar valve, and also that the disturbance caused by the closing of the semilunar valve is propagated as a vibration through the aorta and the carotid artery, at the same rate of speed as the blood pressure waves travel from the heart to the brachial and the radial arteries, as previously determined, namely, 289 inches per second, it follows that the time interval, 0.0215 seconds, as above determined, corresponds to a distance of 6.22 inches travelled by the wave.

On our supposition that the disturbance starts from the closure of the semilunar valve, this time interval represents the difference between the two times of transmission from the semilunar valve to the carotid tambour and from the semilunar valve to the apex tambour at the positions where these tambours were located, the wave disturbance being propagated in different directions from the same origin. Hence the distance, 6.22 inches, represents the difference in the distances from the semilunar valve to the carotid tambour and to the apex tambour.

It should be stated that the length of the tube connecting the tambours with the interference instruments was the same for each, about four feet, and in taking differences the time of travel of a pressure wave through these tubes of the instrument may, therefore, be neglected, although the time is only 0.004 seconds.

The distance from the semilunar valve to the apex tambour may roughly be taken as five inches, and to the carotid tambour as eleven inches, making a difference of six inches in the two distances. This corresponds well with the distance, 6.22 inches, as measured above, and this coincidence, therefore, helps to confirm the supposition that the B-wave-group is caused by the closing of the semilunar valve.

THE PRESPHYGMIC PERIOD.

The two pairs of simultaneous tracings, Nos. 23 and 25, carotid and jugular, show that the B-wave-group appears in both tracings at very nearly the same time. The difference in the location of the jugular and carotid tambours was about two inches, and this distance corresponds to less than 0.01 seconds in the time of travel of the B-wave-group.

In comparing the jugular and the apex tracings, it is necessary to set the jugular back in time to allow for the time of travel of the waves along the vein. If the distance from the mitral valve to the jugular tambour is nine inches, the time required for the waves to travel this distance is 0.031 seconds, or a distance of 0.535 inches in text-figure 1. The jugular tracing No. 23 is represented by the curve 23A in text-figure 1, when it is moved backwards in time 0.031 seconds, and this curve may now be compared directly with the apex tracing No. 24.

It may be objected that the apex tracing should also be moved back about 0.017 seconds, or 0.3 inches, to allow for the time of travel of the liquid wave over the distance, five inches, from the valves to the apex tambour. This should no doubt be done when considering the B-wave-group as above, where a wave is actually transmitted through the blood in the ventricle to the apex tambour; but when considering the presphygmic wave, P, due to a contraction of the ventricle itself, which is almost in contact with the tambour at the apex, no time need be allowed for the travel of the waves. The two curves, apex No. 24 and jugular No. 23A, therefore, stand in their correct positions when the presphygmic period is to be considered.

The presphygmic wave at P in the apex tracing No. 24 is practically identical with this wave as shown at P, text-figure 3, in a former paper.[3] The well known A-wave in the jugular tracing, due to auricular systole, is clearly marked beginning at the point F and finishing at D, 0.12 seconds later. It will be observed that the presphygmic wave, P, in the apex curve, begins at a point X a little before (0.0175 seconds) the close of auricular systole at D. This means that the mitral and tricuspid valves are open at the time the presphygmic contraction sets in, and the immediate effect of this contraction must be to close these large valves, and in so doing a sudden impulse must be transmitted back through the auricles to the veins, and we should expect to find some trace of this shock transmitted to the jugular tambour.

Waves of the nature of a sudden shock such as this will travel along a vein even though there are valves in the vein to prevent the

[3] *Jour. Exper. Med.*, 1911, xiv, 354.

back-flow of a steady current. The impulse will easily pass through the valve without the necessity of the passage of any blood.

The wave we should expect is seen as a very pronounced wave which is marked the M-wave in the jugular record immediately following the crest of the presphygmic wave, P, and is due to the closure of the mitral valve. The crest of this M-wave in the jugular coincides almost exactly with the sharp depression marked M in the apex tracing, and it may therefore be considered that the mitral valve is closed at or near this lowest point in the apex curve, just at the close of the presphygmic wave, P, and also at the beginning of the large wave of ventricular systole, S.

When the real ventricular systole begins, therefore, at M it finds the mitral and tricuspid valves already closed, and there is a firm backing which serves as a good fulcrum to force the blood into the arteries. The closure of the mitral valve at or near the point M leaves the ventricles for a very short time as completely closed chambers; for the pressure has not yet increased sufficiently to open the semilunar valves. In this condition the pressure within the ventricles must rise very rapidly until the semilunars open.

At the point A_0 on the apex curve, just after the sudden rise from the bottom point, M, there is an abrupt jog, which is present in all the apex curves, that is, a sudden change in the direction of the rapid upstroke. It is conjectured that at this point the aortic semilunar valve opens and admits blood to the arteries. That this conjecture is well founded is proved by measuring the time interval between A_0 and the line CC, or the beginning of the upstroke of the carotid; for this interval of time must represent the difference between the times of travel of the blood pressure from the semilunar valve to the carotid tambour, and the wave through the blood in the ventricle to the apex tambour. This time interval we have measured when considering the B-wave-group above, which is due to the closure of this same valve, the opening of which is now being considered. This interval is 0.37 inches in text-figure 1, or 0.0215 seconds, and is very nearly equal to the distance from the point A_0 to the line CC.

The time during which the ventricle is a completely closed chamber is, therefore, probably the interval from the point M to the

point K, where the aortic semilunar valve begins to open, a distance of 0.34 inches or 0.0198 seconds. The valve is completely opened for the flow of blood at A_0. The time during which the chamber of the ventricle is closed is, therefore, approximately 0.02 seconds.

The direction of motion of the valves should also be considered. When the aortic valve opens it does so in a direction away from the apex of the heart and is recorded at K as a slight depression in the apex curve, rather than as an elevation. Similarly, when the mitral valve closes at M, it moves away from the apex and toward the jugular vein, and is recorded as a depression in the former and an elevation at M in the jugular tracing.

In the light of the foregoing description of the events connected with the presphygmic period, it may be said with some confidence that the presphygmic contraction of the ventricle is for the purpose of closing the large mitral and tricuspid valves just before the powerful ventricular systole sets in, in order to prevent the very sudden shock which these valves would receive if they were slammed shut by the sudden impact of blood backed up by the great pressure of systole. This provision makes for the smooth running of the heart without jar or undue strain.

INTRA–STOMACHAL AND INTRA–INTESTINAL INOCULATIONS OF TRYPANOSOME VIRUS WITH TESTS FOR IMMUNITY.*

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Introduction.—In unpublished experiments of Dr. Frederick G. Novy, relapsing fever spirochætæ were introduced directly into the stomachs of rats. As a result of these inoculations, either no infection at all, or only a slight infection was observable. When the rats that had received the virus intra-stomachally were subsequently injected with the same spirochætæ, it was found that all, even those that apparently had not been infected, had acquired a strong immunity.

As rats normally possess no immunity to these spirochætæ, but acquire it after recovering from infection, it was natural to inquire whether the immunity noted in the rats inoculated intra-stomachally, had not arisen as the result of an infection too mild to be detected by microscopical examination.

This question was rendered the more pertinent because rats show very decided differences in the way in which they react towards inoculations of the same spirochætæ. Some have very severe infections and die, while in others the course of the disease is quite mild. On account of the great differences observable in rats infected with spirochætæ, it seemed desirable to repeat the experiments with parasites which show less variation in their action.

For this purpose, pathogenic trypanosomes preserved exclusively in rats seemed to be admirably suited, for when certain species of these parasites infect rats at all, they invariably kill if the animals receive no curative treatment. At the suggestion of Dr. Novy,

* Received for publication, September 5, 1911.

therefore, the work here reported was carried out in the Hygienic Laboratory of the University of Michigan, in the summer of 1908. To Dr. Novy my thanks are due, not only for the problem and virus, but for aid and suggestions during the course of the work.

INTRA-STOMACHAL INOCULATIONS.

Virus.—The trypanosomes employed in the following experiments were those of nagana (*T. brucei*), surra of India (*T. evansi*), dourine (*T. equiperdum*), and caderas (*T. equinum*).

Technique.—Rats alone were employed. In the animals bled for virus, the parasites were, as a rule, 100 or more per field (Zeiss, lens "D," ocular No. 4), in fresh specimens about one corpuscle thick. In securing this virus the rat selected was, under ether anesthesia, bled to death from the heart, the blood was defibrinated, cooled quickly to almost 0° C., and kept at this temperature until used. In most of the experiments the injections of blood were made in less than three hours after it was obtained, and usually within one and one half hours, never later than six hours. In all cases the trypanosomes were still actively motile when injected.

After shaving and disinfecting the skin of the abdomen, a laparotomy was performed under ether anesthesia and the stomach of the rat was drawn through the incision. An injection of one fourth to one half cubic centimeter of richly infected blood was then made directly through the thin cardiac part of the stomach wall. To prevent the escape of blood through the hole in the stomach and to disinfect the wound, a small pledget of cotton that had been thoroughly squeezed out in 2 per cent. lysol was placed on the stomach around the hypodermic needle before this was withdrawn. By raising the cotton from time to time, it could be determined when the puncture in the stomach remained clean. As a rule, the stomach was kept out of the abdominal cavity only a few minutes. When all hemorrhage had stopped, the organ was allowed to slip back into its place and the incisions in the peritoneum, muscles, and skin were sutured with silk.

The results of the intra-stomachal inoculations are given in table I.

TABLE I.

Virus.	Inoculated.	Not infected.	Per cent. not infected.
Surra (*T. evansi*)	5	2	40
Nagana (*T. brucei*)	16	7	44
Dourine (*T. equiperdum*)	13	6	46
Caderas (*T. equinum*)	7	4	57
	41	19	

Table I shows conclusively that trypanosomes may be inoculated directly through the stomach wall without infecting. Of forty-one rats inoculated in this way, nineteen, or nearly 50 per cent., failed to become infected.

The trypanosomes used in these experiments were fully virulent, as was shown by the course of the controls. Most of these controls received minimal inoculations of virus to imitate the infection that might be produced in the experimental animal if only a few trypanosomes succeeded in making their way through the puncture in the stomach into the peritoneum or blood vascular system. For example, one rat received intra-stomachally one half cubic centimeter of nagana blood so diluted that it contained ten trypanosomes per field. As controls on this animal, three other rats were inoculated intra-peritoneally, each with 1 cubic centimeter of diluted nagana blood. The dilutions were 1 to 100,000, 1 to 500,000 and 1 to 1,000,000. All three controls became infected and died, the rat receiving the dilution of 1 to 1,000,000 becoming infected after the others, but one day before the animal inoculated intra-stomachally.

Table I seems to show also that the result of the inoculations varied with the virus employed. Surra of India was apparently the most virulent, infecting 60 per cent. of the rats, and caderas the least virulent, infecting 43 per cent. Nagana and dourine occupied intermediate positions infecting 56 and 54 per cent. respectively.

The trypanosomes most frequently used were those of nagana and dourine. Nagana was selected because of its extreme virulence, and dourine because it is generally thought to possess a characteristic not usually ascribed to nagana, *i. e.*, the ability to pass through unbroken mucous membranes. From the table, however, we see that dourine introduced into the stomach proved slightly less infectious than nagana.

Distended Stomachs.—One point of some practical importance should be mentioned, *i. e.,* that rats having stomachs greatly distended with food are unfavorable for the intra-stomachal inoculations. In every instance in which this organ was markedly distended, even though there was no obvious leakage from it, infection followed the inoculation. Even moderate distention was unfavorable, for only one rat with a stomach in this condition escaped infection.

In order to avoid distended stomachs, it was not sufficient merely to withhold food for a number of hours before operation, for rats often bury food under the saw-dust or shavings in their jars, and dig it up again when hungry. To prevent their doing this, it was necessary to place the experimental animals in jars in which it was known that there was no food, and to keep them there for ten to twenty-four hours before the laparotomy. By this procedure it was possible to obtain stomachs that were empty or nearly so.

Immunity.—It was now of interest to ascertain whether the animals that had escaped infection in table I, had acquired an immunity. To determine this point, all of the rats that had escaped infection were reinjected subcutaneously with small quantities of the virus with which they had been originally inoculated. The object of using small quantities of virus was to detect the presence of even a slight immunity. The trypanosomes injected varied from two per field to less than one in 1,000 fields, and the quantity of suspension inoculated was never more than one fourth of a cubic centimeter and usually only one twentieth of a cubic centimeter. In the beginning the immunity tests were made twenty-eight days after the intra-stomachal inoculations. Subsequently the interval between the original inoculation and the immunity test was shortened to twenty-four, then to twenty, sixteen, and finally to twelve days. The result was the same, however, in every instance. Every animal inoculated became infected and died. Not a trace of immunity was seen.

Hypersensitiveness.—Not only was no immunity observable in the rats that survived the intra-stomachal inoculations, but in the case of surra and nagana there seemed to be a slight hypersensitiveness to infection, for both of the surra rats and five of the seven

nagana rats died before their controls. On the average, the nagana rats died two days earlier than their controls.

INTRA-INTESTINAL INOCULATIONS.

Dourine.—Using technique similar to that employed in the intra-stomachal inoculations, one half cubic centimeter of dourine blood containing ten trypanosomes per field was introduced into the intestines of two rats, in each case within one centimeter of the stomach. Both rats subsequently became infected and died, one as quickly as its control, the other two days later.

Unfortunately, for lack of time, further experiments along this line could not be carried out. No statement, therefore, can be made as to whether animals inoculated intra-intestinally can, even in a small per cent. of the cases, survive this form of injection.

THE VIABILITY OF TRYPANOSOMES IN THE STOMACHS OF RATS.

Attempts to ascertain the influence of the stomach upon trypanosomes introduced into it were made in two ways. (1) The effect upon motility was studied by withdrawing samples of blood from time to time and examining these under the microscope. (2) The retention or loss of virulence was tested by inoculating into normal animals blood that had been withdrawn from the stomach.

Caderas.—With caderas no early tests were made, but two rats inoculated intra-stomachally, each with one half cubic centimeter of blood containing fifty trypanosomes per field, were examined after two and three hours respectively. In the stomachs of neither, however, could a trypanosome be found.

Dourine.—Two rats were inoculated with dourine. Into the stomach of the first rat was introduced one cubic centimeter of blood containing 100 trypanosomes per field. Blood removed from the stomach by needle puncture revealed actively motile parasites during the first forty minutes, but in this interval the number of motile organisms had decreased to one in two fields. By the fiftieth minute the parasites were sluggishly motile and only one was found in ten fields. After this no motile parasites could be detected although a few non-motile organisms could be seen. Eighty min-

utes after the inoculation of the blood, 0.1 cubic centimeter of the stomach contents of this animal was inoculated subcutaneously into another rat, but failed to infect it.

Into the stomach of the second rat, three fourths of a cubic centimeter of blood containing 200 dourine trypanosomes per field was injected. In this rat the parasites remained active for forty-three minutes, but had decreased in this time to five per field. Seven minutes later not a motile trypanosome could be found although the non-motile forms were fairly numerous. Seventy-eight minutes after the introduction of the parasites, 0.1 cubic centimeter of the stomach contents was inoculated subcutaneously into another rat but failed to infect it. From this we see that the virulence of the dourine parasites introduced into the stomach had apparently disappeared in less than eighty minutes.

Nagana.—In the first fifty minutes that followed the intra-stomachal injection of three fourths of a cubic centimeter of nagana blood containing 100 trypanosomes per field, the number of motile parasites had decreased to two per field. After this no motile organisms were seen although a few non-motile trypanosomes were present as late as 105 minutes after the injection of the blood. Some of these were inoculated subcutaneously into a rat but failed to infect it.

Surra.—The parasites of surra were apparently more resistant to the injurious influences of the stomach than were the trypanosomes of the other species. From the stomach of a rat that had received three fourths of a cubic centimeter of blood containing 100 surra trypanosomes per field, samples of blood were withdrawn 5, 15, 30, 50, and 120 minutes after their introduction. In all of these, actively motile trypanosomes were found, although the number of the parasites had decreased from twenty per field at the first withdrawal to one in three fields at the last. From this it is seen that surra trypanosomes may remain actively motile for at least two hours after being placed in the stomach of a rat.

To test the virulence of the trypanosomes, 0.05 cubic centimeter of each of the five samples just described was introduced subcutaneously into rats and in each instance the animal became infected and died. This indicates that surra of India may remain infec-

tious for at least two hours after being introduced into the stomach of a rat.

THE VIABILITY OF TRYPANOSOMES IN THE INTESTINES OF RATS.

Dourine.—One cubic centimeter of blood containing 200 trypanosomes of dourine per field was introduced into the intestine of a rat. Forty-two minutes later blood withdrawn by needle puncture from the intestine contained but one active trypanosome in eight fields, and seventy-five minutes after the introduction of the blood only one actively motile parasite could be found in fifty fields. The animal was now killed by ether, and the intestine was removed and cut into sections one and a half inches long. On examining the section that had been nearest the stomach, two very active parasites were found in searching many fields. The bloody contents of the second section were then taken up in a little salt solution and 0.1 cubic centimeter was inoculated subcutaneously into a rat, but failed to infect it, although a microscopical examination of the fluid in the syringe showed one actively motile parasite in twenty fields. From the intestine of this rat most of the trypanosomes disappeared very rapidly, and the virulence of those that remained was apparently lost in less than an hour and a quarter after their introduction.

Nagana.—One half cubic centimeter of blood containing 100 trypanosomes per field was introduced into the duodenum of a rat, and again the disappearance of the parasites took place with great rapidity. After forty minutes, only two very active flagellates could be found in 100 fields, and after sixty minutes, no motile form of any sort could be discovered. The intestine was then removed and cut into sections an inch and a half long. On slitting open and examining the various sections in order, beginning at the stomach, a few motile trypanosomes were found in the first and third. The other sections were negative.

Surra.—Into the duodenum of a rat, four fifths of a cubic centimeter of blood containing 100 surra trypanosomes per field was injected. On examining the intestine of this animal, seventy-five and ninety minutes later, neither motile nor non-motile parasites

could be discovered. One hundred and fifteen minutes after the injection into the duodenum, it was noted that some blood had made its way into the stomach, so this organ was opened and its contents examined. Rather to my surprise, a number of sluggishly motile trypanosomes were found in the mixture of blood and stomach contents. Here again was shown the exceptional resistance of surra of India to the harmful influence of the stomach.

As far as the writer is aware, experiments exactly like those here described have not been reported, although the feeding experiments of others, *e. g.*, Kanthack, Durham, and Blandford,[1] agree, as far as they go, with the results here recorded, in that they show (1) that trypanosome virus may often be introduced into the alimentary tract without infecting, and (2) that the animals that escape infection do not acquire immunity.

SUMMARY.

In nearly 50 per cent. of the cases, virulent trypanosome virus inoculated directly through the stomach wall of rats failed to infect these animals.

The percentage of infections after inoculation of this kind seemed to vary with the virus. Surra of India proved to be the most virulent, and caderas the least. Nagana and dourine occupied intermediate positions.

The rats that escaped infection showed no trace of immunity when tested with small quantities of the same virus between the twelfth and the twenty-eighth days after the intra-stomachal inoculations.

The majority of the rats tested for immunity with surra of India and nagana seemed to have acquired, instead of an immunity, a certain *hypersensitiveness* to infection. Five of the seven nagana rats died before their controls.

The results of testing the vitality of the trypanosomes of caderas, dourine, and nagana introduced directly into the stomachs and intestines of living rats seem to show that both motility and virulence are usually lost in less than two hours. Surra of India proved

[1] Kanthack, Durham, and Blandford, On Nagana, or Tsetse Fly Disease, *Proc. Roy. Soc.*, 1898–99, lxiv, 100.

exceptionally resistant to the harmful influences of the stomach, for in one case, surra trypanosomes introduced into the *intestines* were found to be sluggishly motile in the *stomach* one hour and fifty-five minutes later, and in another instance, surra parasites injected into the stomach were found, when removed from that organ two hours later, to be actively motile and infectious.

A CHEMO–BIOLOGICAL STUDY OF THE RELATIONS OF PEPSIN TO SO–CALLED ANTI–PEPSIN.*

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During the past five years, because of the limitations of clinical interests, progress in ferment investigation has been somewhat confined to studies concerning anti-trypsin. Relatively little attention has been devoted to the studies of antibodies for the other ferments, although their deep biological significance is apparent. This paper concerns the study of anti-pepsin.[1]

It was recognized that the preliminary step in such an investigation involved the elaboration of a method by which a specific anti-pepsin could be demonstrated. It was considered necessary to use methods similar to those used in serological work for the demonstration of other true antibodies, as it was thought that many of the contradictions in the work already published were due to failure to make use of such technique. The particular importance of careful correlation of chemical and biological technique in the study of pepsin, and an antibody for pepsin, becomes apparent upon consideration of the following:

(1) Several investigations have shown that hydrochloric acid will inhibit the action of immune bodies (Hecker (1)); (2) this acid is necessary for the activation of pepsin; (3) its presence in quantities sufficient for activation of pepsin might inhibit the action of anti-pepsin. Without due consideration of these points in the elaboration of a technique, erroneous conclusions might be drawn, as will be shown in the body of the report.

* Received for publication, August 14, 1911.

[1] Only those articles in the literature will be referred to which have a definite bearing on the points under consideration. The more important articles on anti-pepsin published previous to 1910 are well reviewed by C. Oppenheimer, Die Fermente und ihre Wirkungen, 3d edition, Leipzig, 1910, pp. 270–272.

A clear differentiation should be made between those physical and chemical agents which possess non-specific anti-peptic action, and true specific anti-pepsin.

The following agents have been demonstrated to possess non-specific pepsin inhibiting action: temperature, sunlight (Fermi, Pernossi, Iscovesco), electricity, shaking (Shaklee and Meltzer (2)), filtering through ordinary filter paper (Levy (3)), boric acid (Afanski), carbon dioxid (Schierbeck), glycocoll (Hoffman), leucin (Rosenheim), amino acids (Jastrowitz), accumulated split products, albumoses, peptons, etc. (Pupo-Krüger, Gürber), alkalies (Herzen, Kühne, Langley, Shokiri-Nagayo), trypsin (Langley, Mees), putrefaction (Papasotiriou), salts in general (Dastre, Fabini and Fiori, Wroblewski, Chittenden, Krüger), arsenious acid, inorganic poisons, colloidal metals (Pincussohn), ethyl and propyl alcohol (Laborde), chloroform (Bertels), toluol (Grober), formaldehyde (Bliss and Novy), sugars (Buchner, Sailer and Farr, Ascher), alkaloids (Pawlowsky), tannin (Ascher), saccharin (Matthews).[2]

Of this list of non-specific pepsin-inhibiting agents, the neutral salts have received a large measure of attention. Of these, sodium chlorid has been most thoroughly examined, particularly by Schütz (4) and Levites (5), although the significance of the inhibition of peptic activity by sodium chlorid in relation to the demonstration of a specific anti-pepsin has not been appreciated. The following experiment will serve as an illustration of this inactivation of pepsin by sodium chlorid.

The action of hydrochloric acid was considered to be of such importance that means were taken to free the ferment of as much of this acid as possible. To this end, commercial scale pepsin (Merck) was dissolved in a 25 per cent. glycerin solution, and allowed to stand in the incubator (38° C.) for 24 hours. Ten times the volume of 95 per cent. alcohol was added and the resulting precipitate, after decantation of the supernatant fluid, was dried successively with 95 per cent. alcohol, absolute alcohol, and ether, and then pulverized and placed in a dry, air-tight bottle. This precipitated pepsin was found to be twelve times the strength of the commercial pepsin and almost free from acid. It constituted the stock pepsin with which all experiments were made. Solutions were prepared fresh on the day they were to be used by dissolving the ferment in the given solvent in the proportion of 1 to 10,000, so that the 1 c.c. solution contained 0.0001 gm. of pepsin. This amount was used because after a series of experiments it was found to be the minimum quantity of pepsin which would give definite evidence of proteid digestion, thereby ruling out errors from excess inactive pepsin.

In this experiment, 1 c.c. of an aqueous pepsin solution was placed in each of the nine tubes. To the first three tubes, 2.5 c.c. of 0.85 per cent. sodium chlorid solution were added; to the second three tubes, 2.5 c.c. of distilled water were

[2] This list, with some additions, has been compiled from Oppenheimer, *loc. cit.*, pp. 272–279.

added; the last three tubes served as controls. All tubes were placed in the incubator for 30 minutes. After removal, increasing quantities of N/10 hydrochloric acid were added for activation, and then small portions—about the size of a pea—of carmin fibrin (Grübler). The tubes were again placed in the incubator and observed at intervals for 24 hours. At the end of that time no cloudiness (from digestion of fibrin) and no red coloring (from solution of carmin) of the supernatant fluid had occurred in the tubes containing sodium chlorid. The control tubes with water, and with no further addition, showed marked coloring of the solution in 60 minutes, nearly complete digestion of the fibrin in 18 hours, and complete digestion in 24 hours.

TABLE I.

Tube.	Pepsin in 1 c.c. water.	0.85 per cent. sodium chlorid.	Water.	Incubator for 30 minutes.	N/10 hydrochloric acid.	Carmin fibrin.	10 minutes.	60 minutes.	18 hours.	24 hours.
1	0.0001 gm.	2.5 c.c.			0.5 c.c.	+	0	0	0	0
2	0.0001 gm.	2.5 c.c.			1.0 c.c.	+	0	0	0	0
3	0.0001 gm.	2.5 c.c.			5.0 c.c.	+	0	Trace?	0	0
4	0.0001 gm.		2.5 c.c.		0.5 c.c.	+	0	+	+++	++++
5	0.0001 gm.		2.5 c.c.		1.0 c c.	+	0	+	+++	++++
6	0.0001 gm.		2.5 c.c.		5.0 c.c.	+	0	+	++++	++++
7	0.0001 gm.				0.5 c.c.	+	0	+	+++	++++
8	0.0001 gm.				5.0 c.c.	+	0	+	++++	++++
9	0.0001 gm.				1.0 c.c.	+	0	+	++++	++++
								+	++++	++++

2.5 c.c. of 0.85 per cent. sodium chlorid prevent pepsin in solution in water from digesting carmin fibrin; the addition of N/10 hydrochloric acid in excess does not reactivate ferment. Control tubes alone and with water show complete digestion in 24 hours. 0 = no digestion; +, ++, +++ partial digestion; ++++ = complete digestion.

This inactivation of pepsin by sodium chlorid occurs only when the pepsin is in aqueous solution, with only the trace of acidity contained in the pepsin itself after alcoholic precipitation. If the ferment is dissolved in a weak hydrochloric acid solution, no inactivation by sodium chlorid occurs. Table II shows the results of such an experiment in which the pepsin in aqueous solution (to be referred to as H_2O pepsin) in the first three tubes has been prevented from acting by salt solution; the pepsin in solution $N/10$ hydrochloric acid (to be referred to as $N/10$ pepsin) in the second three tubes digests carmin fibrin promptly. These experiments show the importance of the time of addition of hydrochloric acid in relation to the time of addition of the inactivating sodium chlorid;

one cubic centimeter of $N/10$ hydrochloric acid is sufficient to prevent inactivation when present *before* sodium chlorid (tubes 4, 5, and 6, table II); five cubic centimeters of $N/10$ hydrochloric acid are unable to reactivate pepsin when added after sodium chlorid (tubes 1, 2, and 3, table II).

TABLE II.

Tube.	Pepsin in 1 c.c. water.	Pepsin in 1 c.c. N/10 hydrochloric acid.	0.85 per cent. sodium chlorid.	Incubator for 30 minutes.	N/10 hydrochloric acid.	Carmin fibrin.	10 minutes.	30 minutes.	18 hours.	36 hours.
1	0.0001 gm.		2.5 c.c.		0.5 c.c.	+	0	0	0	0
2	0.0001 gm.		2.5 c.c.		1.0 c.c.	+	0	0	0	0
3	0.0001 gm.		2.5 c.c.		5.0 c.c.	+	0	0	0	0
4		0.0001 gm.	2.5 c.c.		0.5 c.c.	+	+	+	+++	++++
5		0.0001 gm.	2.5 c.c.		1.0 c.c.	+	+	+	++++	++++
6		0.0001 gm.	2.5 c.c.		5.0 c.c.	+	+	+	++++	++++

2.5 c.c. of 0.85 per cent. sodium chlorid prevent pepsin in solution in water from digesting carmin fibrin, in spite of the subsequent addition (in excess) of N/10 hydrochloric acid. Pepsin dissolved in N/10 hydrochloric acid is not prevented from acting by sodium chlorid.

The interest in this phenomenon lies in the fact that physiological salt solution would be considered an anti-pepsin only when pepsin is in aqueous solution, and not when it is in an acid solution. Oguro (6), in a recent article on the quantitative estimation of anti-pepsin in serum, controlled the inhibiting action of horse serum with the non-inhibiting action of sodium chlorid. In this control, four cubic centimeters of a 1 per cent. pepsin solution (Grübler) digested carmin fibrin completely in ten hours. As he did not mention the solvent for pepsin, it was probably distilled water. Therefore, the fact that sodium chlorid did not inhibit must lie in the fact that there was sufficient acid present in the pepsin itself to prevent inactivation. That such was the case seems probable from a comparison of the relative amounts of acidity in the various commercial pepsin preparations.

One gram each of pepsin prepared by Grübler, Armour, Parke, Davis and Company, and Merck, and the precipitated pepsin used in these experiments was dissolved in 100 cubic centimeters of water and titrated with $N/10$ sodium hydroxid; phenolphthalein was used as an indicator. Here the relatively large amount of acid in the

TABLE III.

Comparison of activities of commercial scale pepsin with alcohol precipitated pepsin.

1 gm. of scale pepsin dissolved in 100 c.c. of water. N/10 sodium hydroxid necessary to neutralize.

Grübler	16.7 c.c.
Armour	24.5 c.c.
Parke, Davis	18.2 c.c.
Merck	16.7 c.c.
Alcohol precipitated pepsin	6.9 c.c.

Grübler, as well as in the other commercial preparations, would be sufficient to prevent the salt inactivation, while the small trace of acid in the precipitated pepsin did not interfere. The quantitative estimation of the amount of acid necessary to prevent sodium chlorid inactivation, as well as the question of the inactivating effect of serum which Oguro noted, will be discussed in the second portion of this paper in connection with the study of the blood serum inactivation of pepsin.

There is abundant evidence that in addition to the individual physical and chemical agents capable of pepsin inactivation already discussed, extracts of animal organs, blood serum, various proteids, and the split products of proteid digestion are capable of pepsin inactivation. Schnappauff (7), in 1888, found that fresh liver and muscle extracts destroyed peptic activity. Hahn (8), in 1897, mentions that the addition of dog serum to a pepsin solution prevented the latter from acting. Jacoby (9), in 1906, demonstrated an inhibiting substance in fresh horse serum. Blum and Fuld (10), in 1906, concluded there was an anti-pepsin in normal gastric juice. Jochmann and Kantorowicz (11), in 1908, demonstrated two inhibiting bodies in human serum and edema fluid. Oguro (6), in 1909, discusses a method for the quantitative estimation of anti-pepsin in horse, rabbit, and human serum.

Is the peptic inactivation discussed by these authors due to a specific anti-pepsin present in blood serum, or is it due to some non-specific inhibiting factor? I believe that it is due to the latter, and that it can be satisfactorily explained by the phenomenon of ferment deviation described for some of the other ferments—particularly by Hedin (12) for trypsin. That the deviation of pepsin by serum can be obtained is shown by the following experiment:

0.5 c.c. of beef serum diluted 1 to 5 with salt solution, was added to a series of tubes each containing 0.0001 gm. of precipitated pepsin in 1 c.c. of distilled water; after 30 minutes' incubation, activating acid and carmin fibrin were added. At the end of 24 hours, no digestion of the carmin fibrin had occurred, while the control tubes containing pepsin in N/10 hydrochloric acid showed complete digestion (table IV). A control series with serum diluted with distilled water showed the same inactivation (table V).

TABLE IV.

Tube.	Pepsin in 1 c.c. water.	Pepsin in 1 c.c. N/10 hydrochloric acid.	Beef serum in 1 to 5 sodium chlorid.	Incubator for 30 minutes.	N/10 hydrochloric acid.	Carmin fibrin.	10 minutes.	60 minutes.	18 hours.	24 hours.
1	0.0001 gm.		0.5 c.c.		0.5 c.c.	+	0	0	0	0
2	0.0001 gm.		0.5 c.c.		1.0 c.c.	+	0	0	0	0
3	0.0001 gm.		0.5 c.c.		5.0 c.c.	+	0	0	0	0
4		0.0001 gm.	0.5 c.c.		0.5 c.c.	+	0	0	++	+++
5		0.0001 gm.	0.5 c.c.		1.0 c.c.	+	0	+	+++	++++
6		0.0001 gm.	0.5 c.c.		5.0 c.c.	+	+	++	++++	++++

0.5 c.c. of fresh beef serum in 2.5 c.c. of sodium chlorid prevents pepsin in solution in water from digesting carmin fibrin in spite of the subsequent addition of N/10 hydrochloric acid. Pepsin dissolved in N/10 hydrochloric acid is not prevented from acting by beef serum; the speed of digestion varies directly with the amount of N/10 hydrochloric acid added. This is an example of ferment deviation by beef serum.

TABLE V.

Tube.	Pepsin in 1 c.c. water.	Pepsin in 1 c.c. N/10 hydrochloric acid.	Beef serum in 1 to 5 water.	Incubator for 30 minutes.	N/10 hydrochloric acid.	Carmin fibrin.	10 minutes.	60 minutes.	18 hours.	24 hours.
1	0.0001 gm.		0.5 c.c.		0.5 c.c.	+	0	0	0	0
2	0.0001 gm.		0.5 c.c.		1.0 c.c.	+	0	0	0	0
3	0.0001 gm.		0.5 c.c.		5.0 c.c.	+	0	0	0	0
4		0.0001 gm.	0.5 c.c.		0.5 c.c.	+	0	++	+++	++++
5		0.0001 gm.	0.5 c.c.		1.0 c.c.	+	0	+++	++++	++++
6		0.0001 gm.	0.5 c.c.		5.0 c.c.	+	+	+++	++++	++++

0.5 c.c. of fresh serum in 2.5 c.c. of distilled water prevents pepsin in solution in water from digesting carmin fibrin in spite of the subsequent addition of N/10 hydrochloric acid. Pepsin control dissolved in N/10 hydrochloric acid is not prevented from acting by beef serum. Sodium chlorid solution was not used in serum dilution because of its property of causing pepsin inactivation.

In these experiments, the inactivation of pepsin by serum occurred when pepsin was in aqueous solution, and not in *N*/10 hydrochloric acid. To study whether this deviation would occur, not only

in aqueous solution, but also in the presence of smaller amounts of acid, a technique was employed which would allow of quantitative measurements. This question took on an added interest, for in the event that a small quantity of acid would permit deviation, it might be possible that this degree of acidity would be sufficient to activate pepsin, and at the same time, permit anti-pepsin to act. The method consisted in the nitrogen determination of the non-coaguable proteid after the method described by Opie (14).

0.0001 gm. of pepsin dissolved in the solution to be tested, was placed in each of a series of Erlenmeyer flasks. Then 0.5 c.c. of beef serum, the inhibiting action of which was to be tested, in 1 to 5 sodium chlorid solution was added. After 60 minutes' incubation, 2 c.c. of 2 per cent. hydrochloric acid,[3] 5 c.c. of inactivated beef serum (heated to 75° C. for 30 minutes) in 1 to 5 sodium chlorid solution, and 1 c.c. of toluol were added, and the tubes put in the incubator for 48 hours.

After removal of the coagulable proteids by filtration, nitrogen determinations were made after the method of Kjeldahl, the results being shown in cubic centimeters of $N/10$ sulphuric acid necessary for neutralization. Table VI shows the results of a preliminary series of five flasks with this technique; the results are the same as in the carmin fibrin method.

TABLE VI.

Flask.	Pepsin in 1 c.c water.	Pepsin in 1 c.c. N/10 hydrochloric acid.	Beef serum, 1 to 5 sodium chlorid.	Incubator for 30 minutes.	Hydrochloric acid.	Inactivated beef serum 1 to 5 sodium chlorid.	Toluol.	Kjeldahl N/10 sulphuric acid.
1	0.0001 gm.		0.5 c.c.		2 c.c.	5 c.c.	1 c.c.	6.0 c.c.
2	0.0001 gm.				2 c.c.	5 c.c.	1 c.c.	38.2 c.c.
3			0.5 c.c.		2 c.c.	5 c.c.	1 c.c.	7.0 c.c.
4		0.0001 gm.	0.5 c.c.		2 c.c.	5 c.c.	1 c.c.	39.5 c.c.
5		0.0001 gm.						32.4 c.c.

0.5 c.c. of fresh beef serum in 2.5 c.c. of sodium chlorid prevents pepsin in aqueous solution from acting; when pepsin is dissolved in N/10 hydrochloric acid, digestion is not prevented.

[3] This activating acid was added to bring the acidity of the total mixture to 0.2 to 0.3 per cent., the degree best suited for peptic digestion. It was necessarily added *after* the serum had been in contact with the ferment, otherwise it would not only have prevented deviation because of the large excess of free hydrochloric acid but would probably have destroyed any true anti-ferment present just as acid destroys other antibodies. Hecker, *loc. cit.*, found that hydrochloric acid in strength greater than 0.45 c.c. of N/100 solution prevented the action of hemolytic complement, that 0.4 c.c. gave strong, and 0.35 c.c. complete hemolysis.

A series of flasks was then prepared to study the minimum amount of acidity which would permit pepsin deviation. Control flasks containing inactivated serum were included.

TABLE VII.

Flask.	0.0001 gm. pepsin in 1 c.c. hydrochloric acid.	Fresh beef serum in 1 to 5 sodium chlorid.	Inactivated beef serum.		Hydrochloric acid.	Inactivated beef serum.	Toluol.	N/10 sulphuric acid.
1	N/10	0.5 c.c.		Incubator for 60 minutes.	2 c.c.	5 c.c.	1 c.c.	28.5 c.c.
2	N/10		0.5 c.c.		"	"	"	27.0 c.c.
3	N/20	0.5 c.c.			"	"	"	22.5 c.c.
4	N/20		0.5 c.c.		"	"	"	22.2 c.c.
5	N/30	0.5 c.c.			"	"	"	20.2 c.c.
6	N/30		0.5 c.c.		"	"	"	18.5 c.c.
7	N/40	0.5 c.c.			"	"	"	24.4 c.c.
8	N/40		0.5 c.c.		"	"	"	25.3 c.c.
9	N/50	0.5 c.c.			"	"	"	17.0 c.c.
10	N/50		0.5 c.c.		"	"	"	16.5 c.c.
11	N/60	0.5 c.c.			"	"	"	22.6 c.c.
12	N/60		0.5 c.c.		"	"	"	3.5 c.c.
13	N/70	0.5 c.c.			"	"	"	3.5 c.c.
14	N/70		0.5 c.c.		"	"	"	3.0 c.c.
15	N/80	0.5 c.c.			"	"	"	3.4 c.c.
16	N/80		0.5 c.c.		"	"	"	2.1 c.c.
17	N/90	0.5 c.c.			"	"	"	3.2 c.c.
18	N/90		0.5 c.c.		"	"	"	2.9 c.c.
19	N/100	0.5 c.c.			"	"	"	4.0 c.c.
20	N/100		0.5 c.c.		"	"	"	2.5 c.c.
21	N/200	0.5 c.c.			"	"	"	3.2 c.c.
22	N/200		0.5 c.c.		"	"	"	3.0 c.c.
23	N/500	0.5 c.c.			"	"	"	2.5 c.c.
24	N/500		0.5 c.c.		"	"	"	2.4 c.c.
25	N/10				"	"	"	16.5 c.c.
26		0.5 c.c.			"	"	"	3.0 c.c.
27			0.5 c.c.		"	"	"	2.5 c.c.
28	N/10				"	"	"	3.7 c.c.

0.5 c.c. of inactivated beef serum in 2.5 c.c. of 0.85 per cent. sodium chlorid prevents 0.0001 gm. of pepsin in 1 c.c. of N/60 hydrochloric acid from digesting inactivated beef serum. 0.5 c.c. of fresh beef serum does not prevent pepsin in N/60 hydrochloric acid from acting, but it does prevent pepsin in N/70 hydrochloric acid and pepsin in all greater dilutions up to N/500 hydrochloric acid.

In flask 12, deviation occurred in the presence of *N*/60 hydrochloric acid with 0.5 of a cubic centimeter of inactivated serum; flask 13 showed deviation in the presence of *N*/70 hydrochloric acid with 0.5 of a cubic centimeter of fresh serum.

This experiment showed further that pepsin in *N*/60 hydrochloric acid was deviated by 0.5 of a cubic centimeter of inactivated

serum, but not by 0.5 of a cubic centimeter of fresh serum (flask 11). This speaks strongly against the fact that the inactivation was caused by a specific anti-pepsin, for the anti-ferment would have to be not only thermostabile, but activated by heat. It speaks also against the fact that the hypothetical anti-pepsin may have been destroyed by acid, for in flask 11 pepsin was active, in flask 12 it was inactive, the acidity, *N*/60, remaining constant.

Another series was prepared to study quantitatively the amount of serum capable of causing deviation in a given strength of acid.

TABLE VIII.

Flask.	0.0001 gm. pepsin in 1 c.c. hydrochloric acid.	Fresh beef serum.	Inactivated beef serum.	0.85 per cent. sodium chlorid.	Hydrochloric acid.	Inactivated beef serum.	Toluol.	N/10 sulphuric acid.
1	N/200	0.5 c.c.			2 c.c.	5 c.c.	1 c.c.	4.0 c.c.
2	"		0.5 c.c.		"	"	"	2.8 c.c.
3	"	0.4 c.c.		0.1 c.c.	"	"	"	3.2 c.c.
4	"		0.4 c.c.	0.1 c.c.	"	"	"	2.2 c.c.
5	"	0.3 c.c.		0.2 c.c.	"	"	"	3.2 c.c.
6	"		0.3 c.c.	0.2 c.c.	"	"	"	3.3 c.c.
7	"	0.2 c.c.		0.3 c.c.	"	"	"	2.6 c.c.
8	"		0.2 c.c.	0.3 c.c.	"	"	"	3.0 c.c.
9	"	0.1 c.c.		0.4 c.c.	"	"	"	20.0 c.c.
10	"		0.1 c.c.	0.4 c.c.	"	"	"	15.1 c.c.
11	"	0.08 c.c.		0.42 c.c.	"	"	"	17.7 c.c.
12	"		0.08 c.c.	0.42 c.c.	"	"	"	15.7 c.c.
13	"	0.06 c.c.		0.44 c.c.	"	"	"	16.9 c.c.
14	"		0.06 c.c.	0.44 c.c.	"	"	"	15.4 c.c.
15	"	0.04 c.c.		0.46 c.c.	"	"	"	16.7 c.c.
16	"		0.04 c.c.	0.46 c.c.	"	"	"	18.2 c.c.
17	"	0.02 c.c.		0.48 c.c.	"	"	"	15.8 c.c.
18	"		0.02 c.c.	0.48 c.c.	"	"	"	17.3 c.c.
19	"				"	"	"	8.8 c.c.
20	"			2.5 c.c.	"	"	"	10.6 c.c.
21	"			0.5 c.c.	"	"	"	12.4 c.c.

Decreasing amounts of fresh and inactivated beef serum prevent 0.0001 gm. of pepsin in 1 c.c. of N/200 hydrochloric acid from digesting inactivated beef serum. The minimal quantity of fresh and inactivated serum which will prevent N/200 pepsin from acting is 0.2 c.c. All lesser quantities will not inactivate.

Decreasing amounts of fresh and inactivated beef serum were found capable of deviating pepsin in *N*/200 hydrochloric acid as low as 0.2 of a cubic centimeter of fresh and inactivated serum. All lesser quantities to 0.02 of a cubic centimeter did not inactivate. These experiments demonstrate a quantitative ferment deviation by

fresh and inactivated serum; the point of deviation depends on the relative amounts of acid and serum present. This relation is fairly constant.

Table IX contains the results of a number of experiments in which the minimum quantity of serum capable of deviating ferment in a given acidity was determined.

TABLE IX.

Summary of minimum quantities of fresh and inactivated beef serum capable of deviating pepsin in solution in progressively weaker hydrochloric acid solutions.

0.0001 gm. pepsin in 1 c.c.	Minimum quantity of fresh beef serum capable of deviating pepsin in a given dilution of hydrochloric acid.	Minimum quantity of inactivated beef serum capable of deviating pepsin in a given dilution of hydrochloric acid.
N/60 hydrochloric acid	0.5 c.c.	0.5 c.c.
N/70 hydrochloric acid	0.5 c.c.	0.5 c.c.
N/80 hydrochloric acid	0.4 c.c.	0.4 c.c.
N/90 hydrochloric acid	0.4 c.c.	0.4 c.c.
N/100 hydrochloric acid	0.4 c.c. to 0.3 c.c.	0.4 c.c. to 0.3 c.c.
N/200 hydrochloric acid	0.2 c.c.	0.2 c.c.
N/500 hydrochloric acid	0.1 c.c.	0.08 c.c. to 0.06 c.c.
N/1000 hydrochloric acid	0.01 c.c.	0.01 c.c. to 0.008 c.c.

This shows a proportionate decrease in the amount of serum capable of deviating pepsin in progressively weaker hydrochloric acid solution.

The component or components of blood serum responsible for pepsin deviation are as yet unknown. The investigations of the nature of this body, whether in albumin or globulin fractions of serum, whether colloid or lipoid, dialysable, or non-dialysable, are now in progress. That there is sufficient free hydrochloric acid present to allow pepsin to act, were it not firmly bound to the deviating serum, was demonstrated by titrating the free hydrochloric acid and total acidity of five cubic centimeters of the flask mixture before boiling. In twenty-six flasks so examined, the excess free hydrochloric acid contained in five cubic centimeters needed an average of 1.25 cubic centimeters of $N/10$ sodium hydroxid for neutralization (dimethylamidoazo-benzol being used as indicator); the total acidity needed 2.3 cubic centimeters of N/10 sodium hydroxid (phenolphthalein being used as indicator).

What bearing has this phenomenon of pepsin deviation on the

published accounts of anti-pepsin? The inhibiting substance which Jacoby held to be anti-pepsin was demonstrated as follows:

> He immersed small pieces of washed fibrin in an aqueous pepsin solution, then extracted them and found that pepsin had been removed from the solution and had been bound by fibrin. From this union (which he named pepsin-fibrin), pepsin could be released only by a weak alkali solution, not by water or acid. These pepsin-fibrin particles were active, however, and capable of digesting proteid after the addition of hydrochloric acid. He immersed these pepsin-fibrin particles in horse serum, whereupon it was found that the particles were no longer capable of digesting proteid after hydrochloric acid had been added. He concluded that this inactivation was due to anti-pepsin contained in horse serum.

As I have attempted to show, it was probably due to the serum deviation and binding of pepsin after the latter had been released from its fibrin union by the alkalinity of blood serum. Blum and Fuld themselves comment on the similarity of the action of the inhibiting substance in the gastric juice, which they called anti-pepsin, to the inactivation of pepsin by soluble albumin. Jochmann and Kantorowicz attempted to rule out non-specific pepsin absorption by serum, by demonstrating that free pepsin was present in a neutral pepsin serum mixture and could be withdrawn by a particle of coagulated egg albumin placed in the mixture. The fact that the pepsin deviation by serum is a quantitative phenomenon is sufficient explanation for the fact that free pepsin would be found in the absence of sufficient binding serum. The Jochmann-Kantorowicz solution contained forty times the quantity of pepsin that was used in the solutions in this work.

The same considerations apply to the anti-pepsin of Oguro. His suggestion that the acid itself probably injured or destroyed anti-pepsin, thus accounting for the disappearance of the anti-peptic action when pepsin was dissolved in a weakly acid solution, has been shown to be untenable, for $N/60$ hydrochloric acid permitted the inhibiting body in inactivated serum to act (flask 12, table VII). Likewise, the fact that in the later work ferment deviation was demonstrated in acidities as low as $N/200$, $N/500$, $N/1000$, and the fact that other true antibodies, hemolysin, lysin, etc., have been shown to be active in such acidities, speak conclusively against this view.

In summary, then, it appears that a quantitative non-specific pepsin deviation by blood serum is responsible for what has been described as specific anti-pepsin. Can a specific anti-pepsin be demonstrated in the blood serum of normal animals after ruling out and controlling this non-specific pepsin inactivation?

The demonstration of specific anti-pepsin in the blood of various animals was attempted, according to the technique described for controlling pepsin deviation. $N/500$ hydrochloric acid was used as a routine for the solution of pepsin, as this dilution was considered sufficiently low to permit true antibodies to act. Nine different sera were tested; three human sera from a normal, a nephritic, and a syphilitic (Wassermann positive) patient, respectively; six animal sera from dog, cat, guinea pig, beef, normal rabbit, and pepsin-injected rabbit, respectively. The results of these several experiments are summarized in table X.

TABLE X.

Summary of minimum quantities of fresh and inactivated sera from different animals capable of deviation of 0.0001 gm. of pepsin in 1 c.c. of $N/500$ hydrochloric acid.

Results of tables.	Serum obtained from	Minimum quantity of fresh serum capable of deviating 0.0001 gm. of pepsin in N/500 hydrochloric acid.	Minimum quantity of inactivated serum capable of deviating 0.0001 gm. pepsin in N/500 hydrochloric acid.
11	Human, normal.	0.08 c.c.	0.02 c.c.
12	Human nephritis.	0.08 c.c.	0.06 c.c.
13	Human syphilis.	0.08 c.c.	0.06 c.c.
14	Dog, normal.	0.1 +c.c.	0.1 c.c.
15	Cat, normal.	0.1 c.c.	0.08 c.c.
16	Beef, normal.	0.1 c.c.	0.06 c.c.
17	Guinea pig, normal.	0.1 c.c.	0.06 c.c.
18	Rabbit, normal.	0.08 c.c.	0.06 c.c.
19	Rabbit, injected.	0.1 +c.c.	0.08 c.c.

The series contained abundant evidence of pepsin deviation, but in no instance was there evidenced a specific anti-pepsin. The results show, in addition, a number of interesting facts which the earlier work had not brought out. The most striking of these was seen in the flasks containing fresh sera below the point where deviation had occurred. These flasks showed a progressive increase in the amount of proteid digestion as compared with the control flasks

containing no serum; the amount varied inversely with the amount of serum present. For example, in the table containing the results of the tests of rabbit serum after repeated pepsin immunization, control flask 11, without serum, showed a digestion of 10.5 cubic centimeters of $N/10$ sulphuric acid, while flask 9, containing 0.02 of a cubic centimeter of fresh serum, showed a digestion of 21.5 cubic centimeters of $N/10$ sulphuric acid—twice the amount of the control. The explanation for this apparent pepsin activation is still wanting. There is some evidence that the lipoids of the serum may be concerned, for, in a preliminary experiment concerning the action of lecithin on pepsin, an increased proteid digestion was demonstrable. This investigation is still in progress and will be reserved for later publication in conjunction with Dr. Jobling.

A second interesting fact was observed in the increased deviating or binding ability of inactivated serum as compared with fresh serum. This was more marked in the flasks immediately below the point where deviation with fresh serum had occurred; the flasks further down in the series, containing smaller quantities of both fresh and inactivated serum, showed little if any difference. The explanation of this phenomenon is likewise a subject for future investigation.

The exact point at which deviation occurred varied both with the fresh and inactivated serum. Table X contains the summary of the minimum quantities of fresh and inactivated serum capable of deviating pepsin. This shows a variation of from 0.08 of a cubic centimeter to 0.1 of a cubic centimeter for the fresh serum, and from 0.02 of a cubic centimeter to 0.1 of a cubic centimeter for inactivated serum; a greater range for the latter. In each case, the minimum quantity of inactivated serum capable of deviation was below that of fresh serum, the difference lying between 0.02 and 0.06 of a cubic centimeter.

With few exceptions, the results of the various sera tested were remarkably similar. Normal human serum caused deviation with the minimal quantity as compared with the other sera; 0.02 of a cubic centimeter of inactivated serum caused complete inactivation. Normal dog serum caused deviation with the maximal quantity as compared with the other sera; 0.1 of a cubic centimeter of fresh

serum caused complete inactivation. Serum from a rabbit having had repeated pepsin injections showed a slight increase in the minimum quantity of fresh and inactivated serum, as compared with normal rabbit serum. Whether any significance should be attached to this can be told only after a longer period of immunization. The results of the attempts to produce an immune anti-pepsin must likewise be reserved for future publication.

SUMMARY.

1. Fresh and inactivated animal serum under proper conditions will bind pepsin quantitatively in weak acid solution and will prevent it from digesting proteid even after the addition of free hydrochloric acid in excess.

2. This binding and inactivation of pepsin cannot be considered as due to a specific anti-pepsin.

3. The phenomenon has been named pepsin deviation in analogy with the deviation described for other ferments, notably trypsin.

4. The ability of animal serum to deviate pepsin has been responsible for most, if not all, of the published accounts of anti-pepsin.

5. By the use of a technique elaborated to control pepsin deviation, it has been found impossible to demonstrate normal anti-pepsin in the blood serum of the dog, cat, guinea pig, beef, horse, rabbit, and of man.

In conclusion, I desire to thank Dr. Jobling, at whose suggestion the work was undertaken, for valuable advice and criticism.

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A NOTE ON THE ABSENCE OF ADRENALIN IN MALIGNANT RENAL HYPERNEPHROMAS.*

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INTRODUCTION.

After calling attention to the lack of observations or of any real effort to determine whether adrenalin or adrenalin-like blood pressure raising substances are present in malignant renal hypernephromas, Greer and Wells (1) report the results of the examination of two specimens, both of which gave entirely negative results. Examination of the literature at my command has not revealed much, besides the work of Greer and Wells, in the way of experimental evidence on this question. In the course of the discussion of a paper, Biedl (2) called attention to the point that all tumors which, when removed by surgical operation or found at autopsy, lead one to suspect by their location or other characteristics that they have developed from medullary adrenal tissue should be examined by making tests for adrenalin in extracts of the tumors; and that when so tested, tumors arising from chromaffin tissue of the medulla should show a specific tonus-heightening and especially vasoconstricting substance, while those tumors arising from the cortical tissue should show no such substance. Davidsohn (3) in discussing a paper refers to the presence of adrenalin in a metastatic tumor of the omentum primary in the adrenal, but no very convincing experimental evidence is given. Negative results were obtained by Taddei (4) from some tests on a malignant hypernephroma removed from a boy eleven years of age. Suzuki (5) described in a man sixty-two years of age a tumor which appeared to consist of both medullary and cortical substances, but he gave no direct experiments on the adrenal content of either portion of the tumor.

From the above it would seem that a chromaffin tumor can arise from medullary tissue, but not from cortical rests, which are the places of origin of true malignant hypernephromas. Such chromaffin tumors might well be expected to contain adrenalin, but thus far such a content has not been demonstrated. On the other hand, the ordinary malignant hypernephroma originating in cortical tissue has been tested and found to contain no adrenalin.

The present note is a continuation of, and is supplementary to the paper by Greer and Wells.

ORIGIN OF SPECIMENS.

One of the specimens (A) was metastatic tissue from an adrenal tumor resembling in many respects a renal hypernephroma, and was

*Received for publication, July 10, 1911.

obtained from an autopsy performed on November 9, 1909, by Dr. H. Gideon Wells, at Cook County Hospital. The pathological report has been published by Dr. B. F. Davis (6). There was found a large primary tumor above the right kidney with metastases scattered through the body, especially in the bones and lymphatics. A mass of the metastatic glands was taken from the perihepatic region and afterwards tested for adrenalin as described below. The other two specimens were typical hypernephromas. One of them (specimen B) was a fine specimen, in good condition, which was removed by surgical operation at the Presbyterian Hospital, by Dr. Dean D. Lewis, about two hours before I received it. The other (specimen C) was also a good specimen and weighed about 300 grams. It was removed from a Presbyterian Hospital patient by Dr. A. D. Bevan, and a portion of it reached me about two hours after removal. Also from one of Dr. L. L. McArthur's patients who had a malignant hypernephroma, a specimen of blood was obtained (specimen D), and was examined for its adrenalin content.

EXPERIMENTAL RESULTS.

Specimen A.—Adrenalin glands which contain adrenalin turn black on exposure to air, or even in the body if it is allowed to lie for some days after death. Variations in the amount of blood present in the glands, however, would greatly alter their color, for blood itself would turn black under the same conditions. Although this patient had died four days previously, neither the primary tumor nor the metastases showed any considerable darkening either at autopsy or when pieces were allowed to stand exposed to the air for several days.

Twenty-five grams of the tumor were ground up with sand and fifty cubic centimeters of 0.9 per cent. sodium chlorid solution, and allowed to stand for two hours; the mixture was then filtered, and the filtrate divided into two parts. One part was tested by its effect on carotid blood pressure when injected into the femoral vein of a dog, and by its effect on the rabbit uterus strip. The other portion was acidulated slightly with acetic acid and boiled, after which it was filtered and tested by the same methods employed for the unboiled portion.

A small dog was anesthetized with ether and cannulae were inserted by the usual technique; a three-way cannula in the carotid for blood pressure with a mercury manometer, and a cannula in the femoral vein with a burette for the injection of the solution. Repeated injections were made, beginning with small doses (0.5 of a cubic centimeter) and increasing to larger ones (ten cubic centimeters), but no characteristic adrenalin rise in the blood pressure was observed.

When control extracts made from air-dried adrenals, prepared as described above, were used, they gave the adrenalin rise in the blood pressure curve.

Control injections of adrenalin chlorid solution in doses of one to three cubic centimeters of one part in one million, made in 0.9 per cent. sodium chlorid solution, gave a sharp rise in blood pressure showing the usual blood pressure curve.

Rabbit uterus strips (7) were prepared and mounted in Ringer's solution and kept at a temperature of 38° C. with air slowly bubbling through the solution, and allowed to stand until the strips had ceased contracting and had become quiescent. Dilutions of adrenalin chlorid of one part in ten million, one part in five million, and one part in one million, were prepared in Ringer's solution kept at 38° C. with air slowly bubbling through. The irritability of the uterus strips was tested by displacing the pure Ringer's solution containing the different dilutions of adrenalin. If the strips responded well to one part in one million dilution, they were deemed sufficiently irritable to be used for testing; and if they responded well to the higher dilutions they were thought to give a correspondingly more delicate test for the presence of adrenalin. These same strips together with others which were prepared from the same uterus were then used to test the extracts prepared as described above. When this was done it was found that no contraction of the strips was caused by any of the solutions containing various amounts of the hypernephroma extracts.

The extracts prepared as described were allowed to stand in the laboratory at room temperature for twenty-four hours. It was found that no reddish or pinkish coloration occurred in any of the extracts of hypernephroma. But in the extracts of dried adrenal

glands taken from laboratory animals and prepared as already described distinct pinkish coloration occurred. Also similar pinkish coloration always occurred in the solution of adrenalin chlorid in sodium chlorid solution, or in Ringer's solution.

Specimen B.—Fifteen grams of the tumor were taken and ground fine with sand in a mortar with thirty cubic centimeters of Ringer's solution. It was allowed to stand for two hours, and the filtrate injected in various sized doses, under ether anesthesia, into the femoral vein of a small dog which had its carotid artery connected with a mercury manometer. Doses of one cubic centimeter, five cubic centimeters, and ten cubic centimeters were injected without causing any change in the blood pressure except the very slight rise caused by the injection of that amount of fluid. There was no characteristic adrenalin effect obtained, although the trials were repeated until the supply of extract was exhausted.

Extracts of six adrenals, which had been dried in the open air after having been removed from rabbits and dogs previously used in the laboratory, were made by grinding the glands in a mortar with sand and Ringer's solution, allowing the mixture to stand two hours, and filtering it. When injected intravenously the extracts gave a sharp rise in the blood pressure followed by some fluctuations and irregularities, but in general they showed a very good agreement with the typical adrenalin curve.

Another extract was made with fifteen grams more of the tumor, which were ground in a mortar with fifty cubic centimeters of Ringer's solution, allowed to stand for two hours, and were then filtered. These extracts were tested with rabbit uterus strips and the results controlled by using solutions of adrenalin chlorid to see that the uterus strips were sufficiently irritable to respond to adrenalin in dilution of one part in one million.

Uterus strips were prepared from a large rabbit and two of them were mounted in Ringer's solution at 38° C. with air slowly bubbling through the solution. After waiting until the strips had become quiescent, a solution of one part in one million of adrenalin in Ringer's solution at 38° C. was used to displace the pure Ringer's solution of one of the tubes. The strip in that tube at once contracted strongly. The adrenalin solution was then displaced with

fresh Ringer's solution at the same temperature. The strip very soon relaxed again. Then the two strips were used to test the extract of hypernephroma. One cubic centimeter of the extract was poured into the bottom of the tube, which held about twenty-five cubic centimeters, and time was allowed for the extract to be diffused throughout the solution. There was no contraction of the uterus strip. Although the amounts of extracts added were increased to two, five, and ten cubic centimeters until the supply was exhausted, neither strip of uterus showed any contraction.

The remainder of the tumor material was allowed to stand in the open air for several days to see if blackening would occur; but there was no considerable change in color.

Specimen C.—A piece weighing about 150 grams reached me two hours after removal. The technique of the examination of this tumor is not given in detail because it was in all points similar to that described above for the other tumors.

Pieces of this tumor when exposed to the air blackened but slightly, while pieces of normal adrenals which were exposed under the same conditions at the same time blackened very much.

Extracts made as described above, when injected into the femoral vein of a dog, caused no adrenalin-like rise in blood pressure. There was merely a slight temporary upward curve of the blood pressure tracing such as was caused by the injection of so much salt solution. Control extracts made in the same way and at the same time from adrenals from laboratory animals gave the adrenalin rise in the blood pressure curve. Control injections of solutions of adrenalin chlorid showed the usual adrenalin curve.

Extracts of the tumor, when allowed to stand in room temperature, showed no pinkish coloration, while the extracts of adrenal glands of laboratory animals under the same conditions became pinkish in a few hours.

When applied to the rabbit uterus strips, the extracts of the tumor gave no stimulation, while the control extracts of adrenal glands produced strong contractions, and while also the control solutions of adrenalin chlorid, one part in one million, gave powerful contractions.

Specimen D.—The rabbit uterus strip test was applied.

Uterus strips were prepared from a large rabbit and mounted in Ringer's solution with the air slowly bubbling through and with the temperature kept at 38° C. After the preparation had been allowed to stand until the strips had become quiet, dilutions of the blood of the patient varying from one part in one hundred to one part in ten were used to displace the Ringer's solution around the uterus strips. But in no instance was there any contraction of the strips. Controls of adrenalin chlorid solution showed that the strips would respond with a powerful contraction when placed in a solution of one part in one million.

Dilutions of the blood of less than one part in ten were not used because it was thought that if there were a considerable increase above the normal adrenalin content of the blood, a dilution of one to ten ought to give a positive result with the rabbit uterus test; whereas normal blood might give a positive test in the dilution of one to five.

SUMMARY.

The results of these experiments show, in short, that, by the method employed, adrenalin could neither be demonstrated in the specimen of blood, nor in either of the two specimens of malignant hypernephroma, nor in the specimen of metastatic tissue from the adrenal tumor which closely resembled a malignant hypernephroma.

The results agree with those of Greer and Wells in showing that no adrenalin is found in malignant renal hypernephromas; and the further observation is made that no adrenalin content of the blood of a patient with malignant renal hypernephroma could be shown by the rabbit uterus strip test.

Those rare tumors which arise from medullary adrenal tissue might be expected to contain adrenalin; but as pointed out by Greer and Wells, since the ordinary malignant renal hypernephroma arises from adrenal rests which consist of cortical tissue, and since the cortex of adrenal contains no adrenalin, no adrenalin should be expected in the tumors arising from such cortical tissue.

It is a pleasure to express my thanks to Dr. Joseph L. Miller for assisting me in obtaining material, and to Professor Samuel A. Matthews for assistance with the experimental work.

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